AD_____

Grant Number: DAMD17-02-P-1118

TITLE: Mid-Atlantic Microbial Pathogenesis Meeting

PRINCIPAL INVESTIGATOR: R. Martin Roop, Ph.D.

CONTRACTING ORGANIZATION: East Carolina University
Greenville, North Carolina 27858-4354

REPORT DATE: March 2003

TYPE OF REPORT: Final Proceedings

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 2003	Final Proceedings (3 Feb 03 – 5 Feb 03)

4. TITLE AND SUBTITLE
Mid-Atlantic Microbial Pathogenesis Meeting

5. FUNDING NUMBERS
DAMD17-02-P-1118

6. AUTHOR(S)
R. Martin Roop, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
East Carolina University
Greenville, North Carolina 27858-4354

E-Mail: roopr@mail.ecu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

The first Mid-Atlantic Microbial Pathogenesis Meeting was held on February 3-5, 2003 at Wintergreen Resort in Wintergreen, Virginia. One hundred and forty-five (145) principal investigators, postdoctoral fellows and graduate and undergraduate students from institutions in Pennsylvania, New Jersey, Maryland, the District of Columbia, Virginia, North Carolina and Louisiana participated in the meeting. Fifty-five (55) student travel awards of $350 each were made to all of the students and postdoctoral fellows who made oral or poster presentations at the meeting and a portion of the student travel awards were funded by the award from the USAMRMC. Funding from the USAMRMC was also used to support a portion of the travel expenses of the four keynote speakers who participated in the meeting as well as portion of the costs for printing and mailing of announcements and programs for the meeting. Presentations at the meeting covered a wide range of topics in microbial pathogenesis. With regard to the USAMRMC mission, presentation topics included the virulence mechanisms employed by *Bacillus anthracis* and *Brucella abortus*, two agents that represent potential biowarfare threats, and members of USAMRMC-funded research groups were prominent participants in the meeting. The success of the meeting certainly met, and indeed, exceeded the hopes and expectations of the organizing committee in terms of the quality of the presentations and the extent of the participation by students and postdoctoral fellows. A meeting report is being prepared for submission to the *Journal of Bacteriology*. Probably the best measure of the success of the meeting and the overall enthusiasm of the participants, however, is the fact that preparations are already being made for a second Mid-Atlantic Microbial Pathogenesis Meeting to be held in early February of 2004 at Wintergreen Resort.

14. SUBJECT TERMS
Bacterial pathogenesis, microbial pathogenesis

15. NUMBER OF PAGES
104

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

2003 Mid-Atlantic Microbial Pathogenesis Meeting

Wintergreen, VA

The following organizations provided financial support to the 2003 Mid-Atlantic Microbial Pathogenesis Meeting:

Division of Bacterial, Parasitic and Allergenic Products, Center for Biologics Evaluation and Research, FDA

East Carolina University

United States Army Medical Research and Materiel Command

Animal Health and Well-Being Program, United States Department of Agriculture's National Research Initiative

National Institute of Allergy and Infectious Disease, NIH

Abbott Laboratories

PROGRAM

Monday, February 3

3:00 – 5:00 P.M.		**Registration and Mixer (Devil's Grill Restaurant)**
5:00 – 6:45 P.M.		**Dinner (Devil's Grill Restaurant)**

Evening Session
Moderator: Joanna Goldberg

6:45	Tod Merkel	Welcome and Opening Comments
7:00	Jorge Galan	Mimicry as a strategy to modulate host functions by bacterial pathogens
7:45	Wensheng Luo	Analysis of the Function of EspB in Enteropathogenic *E. coli* (E2348/69) by Random Mutagenesis
8:00	Nicole C. Kesty	Enterotoxigenic *Escherichia coli* (ETEC) Vesicles Target Toxin Delivery into Mammalian Cells
8:15	Marian L. McKee	Secretion of enzymatically inactive *Pseudomonas* exotoxin from CHO cells: evidence that mammalian cells can fold and secrete this bacterial toxin in a near native conformation
8:30	Coffee Break	
8:45	Nicholas Carbonetti	Role of Pertussis Toxin in *Bordetella pertussis* Respiratory Tract Infection
9:15	Laura J. Runyen-Janecky	The Role of the *Shigella flexneri pstS* Gene in Intracellular Growth and Phosphate Acquisition
9:30	Nicole A. Judge	Development of an Edible Vaccine for Cattle to Reduce the Spread of *Escherichia coli* O157:H7
9:45	Anastasia Mitchell	Toll-like Receptor-Dependent Activation of NF-κB in Response to Infection of Human Epithelial Cells by *Helicobacter pylori*
10:00	David J. FitzGerald	Bacterial Toxins: Negotiating the Epithelial Barrier

10:30 – 12:00 A.M.	**Tubing Party (The Plunge)**

Tuesday, February 4

7:00 – 8:00 A.M.		**Breakfast (On your own, voucher provided)**
Morning Session		
Moderator: Robert J. Kadner		
8:00	Peggy Cotter	The *Bordetella* Bvg-intermediate Phase: How it's Controlled and What it Does
8:45	Philip Boucher	Affinity Cleavage of Promoter DNA Reveals a Novel Response Regulator-RNA Polymerase Topology
9:00	Carol A Gilchrist	Calcium Modulates Promoter Occupancy by the *Entamoeba histolytica* Ca^{2+}-binding Transcription Factor URE3-BP
9:15	Cheryl Spence	Upregulation of the *Bacteroides fragilis* Starch Utilization Operon During Oxidative Stress
9:30	Edward G. Dudley	AggR Regulates the Transcription of Genes Encoded on a Major Pathogenicity Island of Enteroaggregative *Escherichia coli* 042
9:45	Coffee Break	
10:00	Craig Altier	Effects of Intestinal Fatty Acids on *Salmonella* Virulence
10:30	John McDowell	Analysis of Factor H Binding Proteins of *Borrelia* Species Associated with Lyme Disease
10:45	E. R. Rocha	The Redox Regulation of *Bacteroides fragilis* Ferritin
11:00	Kara D. Jackson	The *psl*-encoded Exopolysaccharide is Required for *Pseudomonas* aeruginosa PAO1 Biofilm Formation
11:15	Ryan G. Kruger	Kinetic Characterization and Substrate Specificity of the *Staphylococcus aureus* transpeptidase, sortase
11:30	William Petri	The Bittersweet Interface of Parasite and Host: Lectin-Carbohydrate Interactions During Human Invasion by the Parasite *Entamoeba histolytica*
12:00 – 1:00 P.M.		**Lunch (On your own, voucher provided)**
1:00 – 4:00 P.M.		**Free Time**
4:00 – 6:00 P.M.		**Poster Session and Mixer**
6:00 – 7:45 P.M.		**Dinner (Devil's Grill Restaurant)**

Evening Session
Moderator: Maria Sandkvist

8:00	John Roth	Adaptive Mutation and Bacterial Evolution
8:45	Lynette J. Crowther	Interactions Among Cytoplasmic and Cytoplasmic Membrane Components of the Type IV Bundle-forming Pilus Assembly Complex of Enteropathogenic *Escherichia coli*
9:00	Jodi Camberg	Characterization of the Type II Secretion Component EpsE
9:15	Coffee Break	
9:30	Maria E. Scott	The Role of EpsM in Polar Assembly of the Type II Secretion Apparatus of *V. cholerae*
9:45	R. Alcantara	*Brucella abortus* Mutants With Growth Defects Under Starvation Conditions are Attenuated in Cultured Macrophages and in Experimentally-infected Mice
10:00	Meta Kuehn	Production and Toxicity of Bacterial Vesicles

Wednesday, February 5

7:00 – 8:00 A.M. **Breakfast (On your own, voucher provided)**

Morning Session
Moderator: Michael Donnenberg

8:00	Craig Roy	Subversion of Host Cell Early Secretory Pathway by *Legionella pneumophila*
8:45	A. K. Pickering	Transposon Mutagenesis of *Bacillus anthracis*: Identification of Macrophage Invasion Loci
9:00	M. W. Valderas	Genetic Analysis of the Role of the *Brucella abortus* Alkyl Hydroperoxide Reductase, AhpC, in Resistance to Hydrogen Peroxide and Virulence
9:15	Susanne J. Bauman	*Pseudomonas aeruginosa* Outer Membrane Vesicles Produced During Exponential Growth Associate With and Activate Lung Epithelial Cells
9:30	J. Gee	An Evaluation of the Role of the Periplasmic Superoxide Dismutase in the Pathogenesis of *Brucella abortus*
9:45	Coffee Break	
10:00	Ann Jerse	A Gonococcal Efflux Pump System Enhances Bacterial Survival in an Animal Model of Genital Tract Infection

10:30	Janne Cannon	Experimental Human Infection with Neisseria gonorrhoeae: Role of Pili and Opacity Proteins in Infection of the Male Urethra
10:45	Leah E. Cole	Swine Inoculated with *Haemophilus ducreyi* Develop Bactericidal Antibodies to Novel Collagen Binding Outer Membrane Protein DsrB
11:00	Eric L. Buckles	Understanding the Role of Multiple Fimbrial Adhesins in Urinary Tract Infections
11:15	Holly Kuzmiak	Genome Heterogeneity in a Bacteriophage isolated from *Bordetella avium.*
11:30	Tom Kawula	What Should be the Basis for an Efficacious Vaccine to Protect Against *Haemophilus ducreyi* Infection?
12:00 – 1:30 P.M.		**Lunch and Close of Meeting (Devil's Grill Restaurant)**

Abstracts

Monday, February 3
Evening Session

ANALYSIS OF THE FUNCTION OF ESPB IN ENTEROPATHOGENIC *E. COLI (E2348/69)* BY RANDOM MUTAGENESIS

Wensheng Luo* and Michael Donnenberg
University of Maryland school of Medicine

Enteropathogenic E. coli (EPEC) is a major cause of infantile diarrhea in developing countries. EPEC forms characteristic "attaching and effacing" (A/E) lesions on enterocytes. EspA, EspB and EspD are secreted proteins encoded by LEE (locus of enterocyte effacement), that are thought to form a translocation apparatus that injects the effector proteins Tir, EspF, EspG, and Map into host cells. Numerous functions have been attributed to EspB, including an essential role in A/E, Tir translocation, and interactions with EspA. Moreover, EspB is translocated into the host cell membrane as well as the cytosol and stably expressed EspB can change the distribution of actin stress fibers in HeLa cells, suggesting it may also play a role in the cytosol of host cells. Since several functions have been attributed to EspB, we sought to determine through random mutagenesis whether different parts of the molecule are responsible for different aspects of EspB function. Several strategies were used to mutate the EspB gene on a plasmid, plasmids carrying mutated EspB were introduced into an EspB deletion mutant and tested for production and secretion of EspB, ability to perform A/E, and ability to translocate EspF into host cells. Thirty random in-frame insertions were generated and tested. Eleven of these mutants, with insertions located after residues 4, 11, 14, 25, 30, 37, 180, 184, 186, 188 and 252 respectively, retain A/E activity. The remaining eighteen insertions and one deletion at 304 were A/E negative. Interestingly, all seven mutants that have insertions after residue 252 are A/E negative, indicating the importance of the C-terminus of EspB. A hemolysis assay showed that eighteen FAS negative mutants had remarkably reduced hemolytic activity. In contrast, 12 mutants that were FAS positive had levels of hemolytic activity comparable to the wild type E2348/69. Two mutants were notable exceptions. One mutant with an insertion at amino acid 180 was FAS positive, but has dramatically reduced hemolytic activity. Conversely, a second mutant with an insertion at amino acid 240 was FAS negative but had full hemolytic activity. Translocation of an EspF-cyaA fusion protein by the tested mutants generally paralleled the results of hemolytic activity assay. The mutant with the insertion after amino acid 240 demonstrated translocation activity greater than that of several mutants that retained attaching and effacing activity. These studies suggest that EspB has a role in attaching and effacing activity apart from its function as a component of the translocation apparatus. Additional experiments are underway to further analyze these and additional mutants in an effort to understand the function of EspB.

ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) VESICLES TARGET TOXIN DELIVERY INTO MAMMALIAN CELLS

Nicole C. Kesty*[1], Kevin M. Mason[1,2] and Meta J. Kuehn[1]
[1]Duke University Medical Center, Department of Biochemistry, Durham, NC 27710
[2]present address: Children's Research Institute, Ohio State University College of Medicine and Public Health, Department of Pediatrics, Columbus, OH 43205

Enterotoxigenic *Escherichia coli* (ETEC) is responsible for traveler's diarrhea and infant mortality in third world countries. ETEC produces heat-labile enterotoxin (LT), which is structurally and functionally similar to cholera toxin (CT). Unlike CT, LT is not found in the soluble extracellular fraction. We investigated the mode of LT entry into eukaryotic cells. Our lab has found that ETEC produces outer membrane vesicles containing LT and that LT is also bound to the surface of vesicles via LPS. We studied the interactions between fluorescently-labeled ETEC vesicles and eukaryotic cells using a quantitative 96 well plate fluorescence assay and confocal microscopy. ETEC vesicles produced morphological changes in Y1 adrenal cells that corresponded to the amount of bound vesicles. The cell-associated fluorescence was inhibited by the addition of monosialganglioside (G_{M1}), a well-characterized LT receptor. Similar results were observed with the human intestinal epithelial cell line HT29. Cell-associated ETEC vesicles colocalized with fluorescently-labeled CT, another G_{M1} ligand. Caveolae are enriched in G_{M1}, thus we investigated the role of caveolae in vesicle entry. Using filipin, an inhibitor of caveolae formation, we observed decreased binding and internalization. In addition, ETEC vesicles co-fractionated with caveolae in density gradients of cell lysates. Therefore, vesicle-mediated internalization via caveolae appears to be one mode of LT entry into cells. The twin-arginine translocase (Tat) machinery was utilized to transport GFP fused to a Tat-signal sequence into the periplasm of ETEC. Periplasmic GFP was packaged into outer membrane vesicles and will be utilized as a fluorescent lumenal marker to determine the extent of vesicle internalization. These studies demonstrate how bacterial vesicles are vehicles for the transmission of toxins into host cells and suggest an important role for bacterial vesicles in disease.

SECRETION OF ENZYMATICALLY INACTIVE PSEUDOMONAS EXOTOXIN FROM CHO CELLS: EVIDENCE THAT MAMMALIAN CELLS CAN FOLD AND SECRETE THIS BACTERIAL TOXIN IN A NEAR NATIVE CONFORMATION.

Marian L. McKee, Cheol H. Yun, and David J. FitzGerald
Biotherapy Section, Laboratory of Molecular Biology, CCR, National Cancer Institute, Bldg 37, 5124, 37 Convent Dr, MSC 4255, Bethesda, MD 20892-4255

Using the Low density lipoprotein Receptor-related Protein (LRP) as its surface receptor, Pseudomonas exotoxin A (PE) binds and enters mammalian cells via receptor-mediated endocytosis. Once in the endosome, the internalized toxin is partially unfolded, cleaved by furin and transported in retrograde fashion to the endoplasmic reticulum (ER). In the ER additional processing results in the reduction of a key disulfide bond and the translocation of the enzymatically active C-terminal toxin fragment to the cytosol. In the cytosol, PE ADP-ribosylates elongation factor 2, which inhibits protein synthesis and results in cell death. To study toxin trafficking, cloned stable cell lines were established by transfecting CHO cells with a plasmid encoding an enzymatically inactive form of Pseudomonas exotoxin, termed PEÆ553. The toxin gene was preceded by an antibody light chain signal sequence that facilitated secretion to the growth media, where toxin accumulated at a concentration of approximately 10 μg/ml. The functionality of secreted PEÆ553 was confirmed in a competitive cytotoxicity assay. By immunoprecipitation, a doublet of toxin-related protein, corresponding to glycosylated and nonglycosylated species, was recovered in both the culture supernatant and cell lysates. When the secreting cells were probed with anti-PE antibodies, prominent endosomal, Golgi and ER patterns were seen. To determine if intracellular PEÆ553 was occupying key portions of the toxin pathway, we challenged cells exogenously with active PE. Cells expressing PEÆ553 exhibited a 3-log resistance to PE but showed no cross resistance to Diphtheria toxin or ricin. The toxin-resistant phenotype was due in part to a disruption of receptor function as evidenced by lack of LRP ligand binding. The remaining resistance was apparently due to the saturation of an unidentified component necessary for intracellular toxin processing.

ROLE OF PERTUSSIS TOXIN IN *BORDETELLA PERTUSSIS* RESPIRATORY TRACT INFECTION

Galina Artamanova and Nicholas Carbonetti*
Department of Microbiology and Immunology, University of Maryland Medical School, Baltimore, MD

In this study we are trying to determine the role that pertussis toxin (PT) plays for infection and colonization of the respiratory tract by *Bordetella pertussis*, using a mouse model. We constructed an in-frame deletion of the structural subunit genes (S1-S3) of PT in a Tohama I (SN) background so that the only difference from the wild type strain is the absence of PT production. Mouse intranasal infection experiments showed that, over a wide range of bacterial inoculum doses, there was a significant (1-2 logs) difference in peak colonization ability between these 2 strains (WT and ΔPT). This significant defect in colonization of the ΔPT strain showed up early (1-2 days) in a time course of infection. However, in mixed infection experiments with the 2 strains, the starting ratio of the strains (10:1, 1:1 or 1:10) was maintained throughout the time course of the infection (up to 15 days post inoculation), indicating that reduced adherence of the ΔPT strain was not responsible for the lower colonization, and suggesting that PT produced by the WT strain benefited all bacteria equally. In support of the latter idea, we found that the colonization level of the ΔPT strain was increased to that of the WT strain by addition of purified PT to the inoculum. In addition, we are analyzing serum antibody responses to these infections, and have found a greater response to several antigens in the ΔPT-infected mice than in the WT-infected mice, despite the lower numbers of bacteria in the ΔPT-infected mice, suggesting that PT may inhibit antibody responses to the bacteria. We hypothesize that the likely role for PT is to disarm antibacterial immune defense mechanisms of the host, particularly the innate immune responses.

THE ROLE OF THE *SHIGELLA FLEXNERI PSTS* GENE IN INTRACELLULAR GROWTH AND PHOSPHATE ACQUISITION.

Angela Kizzee, Shelley M. Payne, and Laura J. Runyen-Janecky.

Entry into the eukaryotic cytoplasm induces the expression of numerous genes in the facultative intracellular pathogen *Shigella flexneri*. One such gene, *pstS*, is predicted to encode a component of a high affinity phosphate acquisition system. In addition to *pstS*, PCR analysis indicated that *S. flexneri* has the *pitA* and *pitB* genes, which are predicted to mediate low affinity phosphate acquisition. We constructed a mutation in the *S. flexneri pstS* gene, and the mutant formed smaller plaques in eukaryotic cell (Henle) monolayers than the parental strain. The mutant exhibited normal production and localization of the *S. flexneri* IcsA protein, which is required for plaque formation. Addition of extra phosphate to the cell culture medium did not restore the ability of the *pstS* mutant to form wild-type plaques on Henle cell monolayers. The *pstS* mutant had the same growth rate as the parental strain in both phosphate-limited and phosphate-replete media. During the first four hours of growth in Henle cells, the growth rates of the *pstS* mutant and the parental strain were similar. We examined the regulation of phosphate-repressed genes in the *S. flexneri pstS* mutant. During growth in high phosphate media, both the *phoA* and *pstS* genes were constitutively expressed in the *pstS* mutant, but not in the parental strain. This suggests that the inability of the *S. flexneri pstS* mutant to form wild-type plaques in Henle cell monolayers may be due to aberrant expression of phosphate-regulated genes.

DEVELOPMENT OF AN EDIBLE VACCINE FOR CATTLE TO REDUCE THE SPREAD OF *ESCHERICHIA COLI* O157:H7

Nicole A. Judge
Uniformed Services University
Department of Microbiology and Immunology

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is the most common infectious cause of bloody diarrhea in the United States, and a sequela of this infection, the hemolytic uremic syndrome (HUS), is the primary cause of acute renal failure in U.S. children. The majority of U.S. cases of EHEC O157:H7 have occurred as a consequence of ingestion of undercooked, contaminated hamburger or produce and/or water contaminated with bovine manure. Both dairy and beef cattle are reported to be asymptomatically and sporadically infected with this organism. The ultimate goal of this investigation is to develop an inexpensive, effective, easily administered vaccine to prevent cattle from becoming infected with *E. coli* O157:H7 so as to block transmission of these organisms to humans. We are using the carboxy-terminal portion of intimin$_{O157}$ of *E. coli* O157:H7 as the immunogen for such a vaccine because: i.) antibodies against this portion of the outer membrane protein adhesin block adherence of the microbe to tissue culture cells (and to the intestinal mucosa of the weaned piglet) and; ii.) mice immunized intraperitoneally (I.P.) with the carboxy-terminal portion of intimin$_{O157}$ make an antibody response that blocks adherence of *E. coli* O157:H7 to tissue culture cells and show a decrease in the duration of colonization when challenged with *E. coli* O157:H7 strain 86-24. For these studies we elected to use transgenic tobacco cells that express a plant-optimized carboxy-terminal portion of intimin$_{O157}$ as an oral vaccine and tested numerous vaccinatin strategies in a small animal model. Mice fed transgenic plant cells without addition of cholera toxin as an oral adjuvant showed a slight decrease in the duration of colonization, whereas mice fed the transgenic cells with cholera toxin exhibited a greater decrease in the duration of colonization. Mice immunized I.P. with the carboxy-terminal portion of intimin$_{O157}$ partially purified from the transgenic plant cells, then boosted by feeding homologous plant cells showed the greatest decrease (statistically significant when compared to unimmunized mice) in the duration of colonization. Mice fed non-transgenic tobacco cells did not produce an intimin$_{O157}$ –specific immune response and did not exhibit decreased colonization. These results suggest that using intimin$_{O157}$ as an oral vaccine or a vaccine boost in calves is promising since our studies have shown that mice fed or boosted with transgenic plant cells expressing the carboxy-terminal portion of intimin$_{O157}$ make an intimin$_{O157}$ –specific immune response and are not colonized to the same extent as unimmunized mice.

TOLL-LIKE RECEPTOR-DEPENDENT ACTIVATION OF NF-KB IN RESPONSE TO INFECTION OF HUMAN EPITHELIAL CELLS BY *HELICOBACTER PYLORI.*

Anastasia Mitchell, Guolian Li, Ann Marie Fitzmaurice, Michael F. Smith, Jr., and Joanna B. Goldberg.

Helicobacter pylori infection causes gastritis and has been strongly associated with duodenal and peptic ulcers, adenocarcinoma, Non-Hodgkin's lymphoma of the stomach, and MALT lymphomas. Interleukin-8 (IL-8) is a major contributor to the pathogenesis of *H. pylori* infection, and *in vitro* infections have indicated that activation of NF-kB is required for induction of IL-8 in response to *H. pylori*. In this study, we examined the role of the innate immune system in *H. pylori* pathogenesis, specifically, the role of the Toll-like Receptors (TLR). Using a luciferase reporter assay for NF-kB activation, we studied the activation of NF-kB in human epithelial kidney cells (HEK293) transfected with TLR2, TLR4, or TLR5, in the presence of their respective assessory molecules. Upon infection with *H. pylori* 26695, NF-kB was activated in cells transfected with TLR2 or TLR5, but not TLR4. Consistent with the HEK293 experiments, *H. pylori*-induced NF-kB activation was decreased in MKN45 human gastric epithelial cells transfected with dominant negative versions of TLR2 and TLR5 but not TLR4. IL-8 production by HEK293 cells was increased in TLR2 transfected HEK293 cells but not TLR4 or TLR5 transfected cells. Highly purified lipopolysaccharide (LPS) from *H. pylori* activated NF-kB via TLR4 in HEK293 cells. Crude *H. pylori* flagellin activated NF-kB in HEK293 cells transfected with TLR5. These studies indicate that human epithelial cells recognize and respond to infection by intact *H. pylori*, at least in part, through TLR2 and TLR5, and that *H. pylori* flagellin and LPS are able to stimulate NF-kB activation in a TLR-dependent manner.

BACTERIAL TOXINS: NEGOTIATING THE EPITHELIAL BARRIER.

David Fitzgerald[1], Randall Mrsny[2,3] and Marian McKee[1].
[1]Laboratory of Molecular Biology, CCR, NCI, NIH, DHHS.
37 Convent Dr, Bldg 37/5124, Bethesda, MD 20892.
[2]Genentech, Inc. [3]Current address Trinity Biosystems, Palo Alto, CA.

Epithelia represent a natural barrier to microbial invasion. However, many pathogens secrete virulence factors that subvert this barrier without overtly destroying it. Toxins released on the apical side of epithelia can apparently translocate to the basolateral side and damage underlying tissues. Examples include: Botulinum toxin, Staphylococcal enterotoxins, diphtheria toxin, exotoxin A (from Pseudomonas), cholera toxin and others. To investigate toxin translocation, we have employed 2 model systems. When exotoxin A was applied directly to mouse tracheal epithelium, there was damage to submucosal tissues, liver and spleen and active toxin could be recovered in serum samples. However, upon histological examination, there was very little damage to the tracheal epithelium itself. This result was then recapitulated in a model tissue culture system. Caco-2 cells exhibited a toxin-resistant phenotype when grown as a polarized monolayer on collagen-coated transwells but were quite toxin sensitive when grown as dispersed cultures. In attempting to understand this latter observation, we have prepared RNA from cells grown under either condition and examined gene expression patterns using cDNA microarrays. In addition, we have performed a number of biochemical characterizations of cell fractions from polarized and dispersed cells. We propose that polarized epithelia protect themselves from toxin-mediated damage by allowing translocation of intact toxin molecules to subepithelial tissues.

Tuesday, February 4
Morning Session

AFFINITY CLEAVAGE OF PROMOTER DNA REVEALS A NOVEL RESPONSE REGULATOR-RNA POLYMERASE TOPOLOGY

Philip Boucher, Ann Maris, Mei-Shin Yang, Scott Stibitz
CBER/FDA, Bethesda, MD 20892.

We have investigated DNA binding of the response regulator BvgA and the RNA polymerase alpha subunit C-terminal domain (alpha-CTD) at the *fha* promoter of *Bordetella pertussis* through the use of affinity cleavage reagents created by conjugation with FeBABE. These studies have revealed that three dimers of BvgA, formed by the head-to head association of monomers, bind with a two-helical turn periodicity, covering one face of the DNA helix from the inverted-heptad primary binding site centered at –88. 5 to the –35 region. Modeling studies revealed that the orientation of BvgA monomers within the dimers is the same as that recently demonstrated by X-ray crystallographic methods for the C-terminal domain of the related response regulator NarL bound to DNA. Cleavage patterns generated by RNAP containing FeBABE conjugated to its alpha subunit C-terminal domain showed that binding of this domain is linearly coincident with binding of the BvgA dimers, but to a different helical face. These results reveal a novel mode of interaction between RNAP alpha-CTD and a transcriptional activator.

CALCIUM MODULATES PROMOTER OCCUPANCY BY THE *ENTAMOEBA HISTOLYTICA* Ca^{2+}-BINDING TRANSCRIPTION FACTOR URE3-BP

Carol A Gilchrist[1], Megan Leo[1], C. Genghis Line[1], Barbara J. Mann[1,2], William A. Petri Jr [1,2,3]
Departments of Internal Medicine[1], Microbiology[2], and Pathology[3], University of Virginia, Charlottesville, VA, USA

The early branching eukaryote *Entamoeba histolytica* is a human parasite that is the etiologic agent of amebic dysentery and liver abscess. Only one of every 10 infections leads to disease and the parasite and host factors that control the outcome of infection are not well understood. The mechanisms that control the changes from asymptomatic colonization to invasive amebiasis may involve changes in the expression of proteins involved in *E. histolytica* pathogenicity. There has been considerable divergence in the mechanisms of transcription of the early branching *E. histolytica* from later branching eukaryotes such as *H. sapiens* and *S. cerevisiae*. To increase our understanding of transcriptional regulation in this organism we investigated the mechanisms of transcriptional control of a well-characterized virulence protein, the galactose- and N-acetyl-D-galactosamine-inhibitable lectin (Gal/GalNAc-inhibitable lectin), which is essential for parasite adherence and contact-mediated cytolysis. The *hgl5* gene of *E. histolytica* is negatively regulated through the URE3 DNA motif TATTCTATT. A yeast-one-hybrid screen was used to identify an *E. histolytica* cDNA encoding a protein (URE3-BP) that recognized this DNA motif. The *Entamoeba histolytica* URE3-BP protein was previously identified by virtue of its ability to bind to the upstream regulatory element 3 (URE3) sequence of the Gal/GalNAc lectin *hgl5* gene. Two EF-hand motifs, (correlated with the ability to bind calcium), are present in the amino acid sequence of URE3-BP. Mutation of the second EF hand motif in URE3-BP resulted in the loss of calcium inhibition of DNA binding as monitored by electrophoretic mobility shift assay. Chromatin immunoprecipitation assays revealed that URE3-BP was physically bound to the *hgl5* promoter *in vivo*. Promoter occupancy was abolished when the intracellular calcium level was increased (by manipulating extracellular calcium concentrations). Calcium concentration-dependent binding of URE3-BP to the *E. histolytica* ferredoxin (*fdx*) promoter that contains a functional URE3 DNA motif was also observed. These results suggest that calcium regulates the ability of URE3-BP to bind to URE3 *in vitro*, and to URE3-containing promoters *in vivo*. Modulation of URE3-BP sequence-specific recognition of DNA by intracellular calcium levels may represent a novel mechanism of control of gene expression.

Direct regulation of a transcription factor via calcium binding is very unusual although calcium is an important intracellular messenger in eukaryotes. We are investigating the role of URE3-BP in *E. histolytica* calcium-dependent gene expression.

UPREGULATION OF THE *BACTEROIDES FRAGILIS* STARCH UTILIZATION OPERON DURING OXIDATIVE STRESS.

Cheryl Spence* & C. Jeffery Smith
Dept. Microbiology & Immunology, Brody School of Medicine,
East Carolina University. Greenville, NC. 27858

The obligate anaerobe *Bacteroides fragilis* is a commensal organism of the human gastrointestinal (GI) tract. *B. fragilis* is also a successful opportunistic pathogen, commonly isolated from anaerobic infections such as abdominal abscesses and post-operative wound infections. This bacterium's ability to cause disease has been attributed to a broad array of virulence factors. In particular, its high survival rate outside of the anaerobic confines of the GI tract has been attributed to a mechanism of aerotolerance and its ability to survive during oxidative stress. This oxidative stress response (OSR), is considered a major virulence factor of *B. fragilis*. As an anaerobe, *B. fragilis* does not divide in the presence of oxygen, however, under aerobic conditions it does synthesize new RNA and proteins for at least 48 hours of oxygen exposure. For *B. fragilis* to maintain such an extensive response mechanism to oxidative stress, it must require a source of energy. Reported here are studies on an outer membrane protein (OMP) whose expression is upregulated during exposure to oxidative stress. This OMP has significant homology to SusC, an OMP involved in starch utilization in *B. thetaiotaomicron*, and to RagA, a major surface antigen of *Porphorymonas gingivalis*. The gene encoding this OMP is one of four genes comprising a 9 kb operon, that has been designated oxygen-induced starch utilization (*osu*). A mutant was constructed by insertional inactivation of the OMP-encoding gene, and phenotypic analysis determined that this mutant was severely impeded in its ability to utilize starch or glycogen. The gene *osuA* encodes an OMP that has putative porin function. The second gene in the operon encodes a protein with homology to another OMP that may serve to stabilize the porin in the membrane. The *osuC* gene encodes a protein of unknown function, but may play a role in the processing of starch, and the final gene *osuD*, potentially encodes an α-amylase which is involved in the breakdown of starch. This operon was upregulated during exposure to molecular oxygen as evidenced by Northern blot analysis, and it was transcribed as a polycistronic message as shown by RT-PCR. This *osu* upregulation appeared to be OxyR-independent, as evidenced by Northern blot analysis of an OxyR mutant, suggesting another level of control in the OSR. In the opposite orientation, directly upstream of this operon is a gene encoding a putative regulatory protein, (*orfR*). Interestingly, a dominant 7 kb message is upregulated in response to oxygen exposure, but a less abundant 9 kb mRNA species is also seen. When Northern analysis is performed using cells grown with starch as the sole carbon source, the 9 kb message is the predominant mRNA. This suggests different expression of the 7 kb and 9 kb transcripts as a result of oxygen stress or carbon source. A putative transcriptional terminator is located between *osuC* and *osuD*. Studies using the *osuA* mutant and construction of a mutant defective in the putative regulator are currently being undertaken to further define the regulatory control of this operon, and to determine its functional role in the OSR.

AGGR REGULATES THE TRANSCRIPTION OF GENES ENCODED ON A MAJOR PATHOGENICITY ISLAND OF ENTEROAGGREGATIVE *ESCHERICHIA COLI 042*.

Edward G. Dudley* and James P. Nataro
University of Maryland-Baltimore Center for Vaccine Development.

Enteroaggregative *Escherichia coli* (EAEC) is an emerging enteropathogen associated with infantile diarrhea in developing countries and has also been associated with food-borne diarrhea in developed countries. This *E. coli* pathotype is defined by aggregative adherence (AA) to HEp-2 cells, where bacteria display adherence to the cell surface and also to the intervening substratum in a stacked-brick configuration. The AA phenotype is associated with the presence of a plasmid 60 to 65 MDa in size and with the expression of one of two distinct aggregative adherence fimbriae (AAF/I and AAF/II). We have previously shown that an AraC homolog designated AggR is required for expression of both AAF/I and AAF/II. To identify other genes under AggR control, 2-dimensional gel electrophoresis was used. Eleven protein spots found in the proteome of wild-type EAEC strain 042 were absent in the proteome of an isogenic *aggR*-mutant. The gene encoding the most prominent protein was cloned, and predicted to encode an 18.2 kDa protein. Database searches indicate neither the gene, designated *aaiA*, or its corresponding protein product have been described in other organisms. Colony hybridizations using a probe synthesized from an internal portion of *aaiA* suggest this gene is conserved in approximately 66% of EAEC strains. Regions flanking *aaiA* were isolated from a genomic DNA library of 042, and initial sequencing suggests *aaiA* is encoded on a pathogenicity island of >60 kb that is not present in *E. coli* K-12. Analysis of mRNA indicates *aaiA* is cotranscribed with at least three other genes, suggesting AggR directly or indirectly regulates a polycistronic message. These and surrounding genes are observed in a number of animal and plant pathogens, suggesting they function in a conserved pathogenic mechanism. Characterization of a strain lacking *aaiA* is in progress.

EFFECTS OF INTESTINAL FATTY ACIDS ON *SALMONELLA* VIRULENCE

Craig Altier[1], Sara D. Lawhon[1], Jonathan G. Frye[2], Mitsu Suyemoto[1], Steffen Porwollik[2], Michael McClelland[2], and Russell Maurer[3]
[1]North Carolina State University, Raleigh, NC, [2]Sidney Kimmel Cancer Center, San Diego, CA, [3]Case Western Reserve University, Cleveland, OH.

As *Salmonella* passes through the intestinal tract of an animal host, it encounters a resident microflora of increasing complexity. The milieu produced by these commensal organisms might allow the pathogen to sense its location within the intestinal tract and to accordingly alter its expression of virulence determinants. An important component of this environment is short chain fatty acids, including acetate, propionate, and butyrate, produced as the result of fermentation of carbohydrates by the colonic microflora. The composition of short chain fatty acids changes through the intestine, and so might provide a means by which *Salmonella* can sense its immediate environment. In the ileum, the primary site of *Salmonella* infection, total short chain fatty acid concentration is relatively low, with acetate predominating. In the colon, short chain fatty acid concentration rises dramatically, with relative increases in the proportions of propionate and butyrate. We show that the short chain fatty acid composition of the ileum, and specifically acetate, promoted epithelial cell invasion through induction of *Salmonella* pathogenicity island 1 (SPI1) invasion genes. Acetate had this effect only at a pH that allowed its accumulation within the bacterial cytoplasm and only with the production of acetyl-phosphate. Additionally, acetate affected invasion only in the presence of the sensor kinase SirA, an inducer of SPI1. We propose that acetyl-phosphate functions by directly phosphorylating SirA, supplanting the need for its cognate sensor kinase, BarA. Conversely, colonic short chain fatty acids conditions, and propionate and butyrate individually, inhibit invasion by repressing these same SPI1 genes. Thus, the production of short chain fatty acids by the resident intestinal microflora can identify for *Salmonella* a productive site for infection, the distal small intestine, but repress invasion once bacteria pass into the large intestine. We have also determined the global effects of short chain fatty acids on *Salmonella* gene expression using DNA microarrays. This genomic analysis showed that, in addition to invasion, ileal conditions induced the type III secretion apparatus of *Salmonella* pathogenicity island 2 (SPI2), required for bacterial survival in macrophages. Therefore, ileal short chain fatty acids appear to induce two functions important to *Salmonella* virulence. As expected, colonic conditions repressed invasion genes, but also those required for flagellar synthesis and chemotaxis, and for maltose and maltodextrin transport. Thus, the changing short chain fatty acid conditions of the intestinal tract provide cues to alter *Salmonella* virulence as well as other functions. Since intestinal short chain fatty acid composition can be manipulated by changes in diet, these signals provide a potential means to alter *Salmonella* virulence.

ANALYSIS OF FACTOR H BINDING PROTEINS OF *BORRELIA* SPECIES ASSOCIATED WITH LYME DISEASE

John McDowell, Duncan Hamilton, Emily Tran, Jill Wolfgang, Sean Metts, Kelley Miller, and Richard T. Marconi

Lyme disease is caused by pathogenic species of the *Borrelia burgdorferi* sensu lato (Bbsl) complex. Of the ten species in this complex, only *B. burgdorferi*, *B. afzelli*, and *B. garinii* have been shown to cause disease in humans. Henceforth these species will be referred to collectively as the Lyme disease spirochetes (LDS). If untreated infection with the LDS is chronic indicating an ability to circumvent the immune response. Recently, it has been demonstrated that some LDS isolates can bind the complement regulatory protein factor H. This study represents a comprehensive analysis of the factor H binding capability of the LDS. To assess binding, whole-cell ELISA and affinity ligand binding formats were applied. The ability to bind factor H differed among species of the Bbsl complex and extensive variation was observed in the number and molecular weight of the binding proteins. These analyses revealed that the serum resistant species, *B. burgdorferi* and *B. afzelii* bind factor H while serum sensitive *B. garinii* do not. The inability of *B. garinii* to bind factor H may explain the tropism of this species for the central nervous system. Of the multiple factor H binding proteins of the LDS, only one, OspE, has been identified to date. To identify the determinants of OspE that are required for factor H binding, N and C terminal truncations of OspE were made and the recombinant proteins were tested for their ability to bind factor H. Deletion of either the N or C-terminus abolished binding indicating that conformational or discontinuous determinants are required for binding. These studies further our understanding of the mechanisms employed by the LDS utilize to evade host immune destruction and maintain chronic infection. The ability to evade immune destruction is a critical component of the natural enzootic cycle of these pathogens and is essential for population maintenance.

THE REDOX REGULATION OF *BACTEROIDES FRAGILIS* FERRITIN.

E. R. Rocha and C. J. Smith.
East Carolina University School of Medicine, Greenville, NC.

The anaerobic bacterium *Bacteroides fragilis* is the most frequent anaerobe isolated from human infections. It is known to require heme for growth, and this can be substituted by protoporphyrin IX and non-heme iron, but overall little is known about iron acquisition and storage in anaerobic bacteria. This is probably because free Fe^{2++} can exist in the absence of oxygen and is likely to be readily available at neutral pH. Furthermore, the absence of oxygen lowers the risk of toxic radical generation. Nevertheless, this may be an over-simplification since we have previously isolated a ferritin (an iron storage protein found in aerobic eukaryotes and prokaryotes) homologue in *B. fragilis* which binds iron under anaerobic conditions. In order to identify the ferritin (*ftn*) gene, oligonucleotides complementary to the conserved regions of other FTN genes were used to PCR amplify a 361 bp *ftn* internal fragment from the *B. fragilis* chromosome. A combination of inverse PCR and marker rescue were then used to obtain the entire gene region. Nucleotide sequence and Southern blot analysis revealed a *ftn* homologue that encodes a protein with 45 to 60 % identity to FTN from bacteria and mammals. Northern blot hybridization and primer extension analysis revealed that *ftn* is transcribed as a monocistronic mRNA of approximately 800 nucleotides and there were two transcription initiation start sites. The *ftn* message increased about 10-fold after oxygen exposure in iron-replete conditions compared to iron-limiting conditions. One promoter was found to be constitutively expressed while the second promoter was responsive to oxidative stress. Induction by hydrogen peroxide and oxygen was greatly reduced in an *oxyR* deletion mutant. This strongly suggests that *B. fragilis ftn* is regulated by both the peroxide response transcriptional activator OxyR and an oxygen-dependent regulator. Moreover, addition of potassium ferricyanide but not ferrous sulfate induced expression of *ftn* mRNA anaerobically suggesting that the oxidation state of iron affected expression of *ftn*. This was further investigated by addition of diamide, a thiol-oxidizing agent, which greatly induced *ftn* mRNA expression suggesting that an unbalanced cellular redox state affects *ftn* expression. Taken together, these data show that under aerobic conditions but not anaerobic conditions the expression of *B. fragilis ftn* was affected by the availability of iron in the oxidized form but not in the ferrous form. Moreover, the oxidative stress response regulation of *B. fragilis ftn* suggests that iron-storage upon oxygen exposure is an important mechanism for protection against metal-induced oxygen radical formation in aerotolerant anaerobic bacteria.

THE *PSL*-ENCODED EXOPOLYSACCHARIDE IS REQUIRED FOR *PSEUDOMONAS AERUGINOSA* PAO1 BIOFILM FORMATION

Kara D. Jackson[1*], M. Parsek[2], M. Starkey[2], G. O'Toole[3], T.J.O. Wyckoff[1], and D.J. Wozniak[1]
[1]Wake Forest University School of Medicine
[2]Northwestern University, and [3]Dartmouth Medical School

In the lungs of cystic fibrosis patients, *Pseudomonas aeruginosa* has been shown to form biofilms, which are collections of aggregate bacteria bound to a surface. Bacteria residing within biofilms usually produce one or more polysaccharides that function to stabilize and reinforce the structure of the biofilm. Historically, alginate has been considered the major exopolysaccharide of *P. aeruginosa* biofilms. In this study, we examined the chemical nature of the biofilm matrix expressed by *P. aeruginosa* strain PAO1. In addition, biofilms were studied with wild type and isogenic alginate mutants and *algD* transcription was monitored over time. The results clearly indicate that alginate biosynthetic genes are not expressed and that alginate is not required for the formation of *P. aeruginosa* PAO1biofilms. This suggests there is at least one other polysaccharide operon involved in mediating biofilm formation. Inspection of the *P. aeruginosa* annotated genome revealed three novel putative polysaccharide loci. One of these loci was chosen for further study and designated *psl*. Genes from this *psl* operon were cloned and reverse genetics employed to generate mutants in *P. aeruginosa* strain PAO1. In static microtiter biofilm assays, these mutants were defective at biofilm initiation when compared with the wild isogenic parental strain PAO1. This impaired biofilm initiation phenotype could be complemented with wild-type sequences and was shown not to be due to defects in flagellar-mediated or twitching motility. These results implicate an as yet unknown exopolysaccharide required for formation of the biofilm matrix. Determining the role of the *psl* encoded exopolysaccharide in biofilm formation will provide insight into the pathogenesis of *P. aeruginosa* in cystic fibrosis and other infections involving biofilms

KINETIC CHARACTERIZATION AND SUBSTRATE SPECIFICITY OF THE *STAPHYLOCOCCUS AUREUS* TRANSPEPTIDASE, SORTASE

Ryan G. Kruger, Brenda A. Frankel, and Dewey G. McCafferty

Sortase enzymes play a key role in the virulence of several Gram-positive bacteria by catalyzing the covalent anchoring of surface protein virulence factors to the bacterial cell wall peptidoglycan. These virulence factors include adhesion proteins such as fibronectin-binding protein and related microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) as well as proteins designed to help the bacteria evade detection by the host immune system (i.e. Protein A). The *Staphylococcus aureus* enzyme SrtA catalyzes a transpeptidation reaction in which the surface-protein substrate is cleaved between the threonine and the glycine of a conserved LPXTG recognition motif, and the threonine carboxyl is subsequently linked, via amide bond formation, to the free amino group of a pentaglycine crossbridge in the peptidoglycan layer. Multiple sortase isoforms along with multiple variations of the LPXTG recognition motif have been found in various Gram-positive bacteria, suggesting that sortase isoforms carry out the cell wall anchoring of unique subsets of virulence-associated proteins. Successful inhibitor design hinges upon understanding the catalytic mechanism of sortase isoforms and decoding the molecular logic of their substrate selectivity. We have cloned, overexpressed, and purified the sortase A isoform SrtA from *S. aureus* and have developed a novel HPLC-based assay to monitor its catalytic activity. Using this assay with the peptide substrate Abz-LPETG-Dap(Dnp), we determined the kinetic parameters of SrtA to be $k_{cat} = 0.27$ sec^{-1} and $K_m = 5.5$ mM. To examine the specificity of SrtA, we synthesized and evaluated a peptide library containing 90 substrates based on the LPXTG motif. We have found that Sortase A is exquisitely specific for the Pro and Gly positions of the LPXTG motif. Although Leu and Thr are kinetically preferred in these positions, alternate beta-branched amino acids are tolerated. The X position, identified as a variable position by sequence comparison studies, exhibited a preference for Met and other hydrophobic residues, although at long reaction times, all amino acids could be efficiently processed. Our studies suggest that SrtA, one of the two sortase isoforms in *S. aureus*, is most likely responsible for attachment of proteins containing the LPXTG sequence. Transition state inhibitors based on this sequence should prove to be interesting candidates for SrtA-selective antivirulence therapeutics.

THE BITTERSWEET INTERFACE OF PARASITE AND HOST: LECTIN-CARBOHYDRATE INTERACTIONS DURING HUMAN INVASION BY THE PARASITE *ENTAMOEBA HISTOLYTICA*.

William A. Petri, Jr.
Departments of Medicine, Microbiology and Pathology, University of Virginia

Entamoeba histolytica, as it name suggests, is an enteric parasite with a remarkable ability to lyse host tissues. However the interaction of the parasite with the host is more complex than solely destruction and invasion. It is at the host:parasite interface that cell-signaling events commit the parasite to (a) commensal, non-invasive infection, (b) developmental change from trophozoite to cyst, or (c) invasion and potential death of the human host. The molecule central to these processes is an amebic cell surface protein that recognizes the sugars galactose (Gal) and N-acetylgalactosamine (GalNAc) on the surface of host cells. Engagement of the Gal/GalNAc lectin to the host results in cytoskeletal reorganization in the parasite. The parasite cytoskeleton regulates the extracellular adhesive activity of the lectin and recruits to the host-parasite interface factors required for parasite survival within its host. If the parasite lectin attaches to the host mucin glycoproteins lining the intestine the result is commensal infection. In contrast, attachment of the lectin to a host cell surface glycoprotein leads to lectin-induced calcium transients, caspase activation and destruction of the host via apoptosis. Finally, trophozoiite quorum sensing via the lectin initiates the developmental pathway resulting in encystment. The crucial cell biologic processes that lead to these divergent paths are the subject of this presentation.

Tuesday, February 4
Evening Session

INTERACTIONS AMONG CYTOPLASMIC AND CYTOPLASMIC MEMBRANE COMPONENTS OF THE TYPE IV BUNDLE-FORMING PILUS ASSEMBLY COMPLEX OF ENTEROPATHOGENIC *ESCHERICHIA COLI*

Lynette J. Crowther* and Michael S. Donnenberg
Division of Infectious Diseases, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland, 21201

Enteropathogenic *Escherichia coli* (EPEC) is a major cause of severe infantile diarrhea, particularly in developing countries. The bundle-forming pilus (BFP) of EPEC is an established virulence factor and has been implicated in adherence to host cells. A fourteen-gene operon, known as the *bfp* operon, located on the EPEC adherence factor plasmid encodes the BFP. Although very little is known about the molecular mechanisms of BFP synthesis, it is believed that many, if not all, of the Bfp proteins compose a molecular machine necessary for biogenesis of functional BFP. Here we have studied interactions between individual components of the assembly complex to provide a more coherent picture of this assembly machine.

We predicted that BfpD, a putative cytoplasmic ATPase and energy supplier in BFP biogenesis, is anchored to the inner membrane by the inner membrane proteins BfpC and/or BfpE. To test this, we intended to use plasmids pLJC1, pRPA314 and pLJC2 (containing *bfpD*, *bfpC* and *bfpE* respectively) to express BfpD alone or together with BfpC or BfpE and study BfpD localization in *E. coli* strain TOP10F'. However, BfpD could not be detected by Western blot analysis of TOP10F' containing pLJC1 and pRPA314 or pLJC2. In contrast, BfpD was easily detected in TOP10F' containing pLJC1 alone or pLJC1 and control plasmid pTrcphoA. Competitive RT-PCR showed that the presence of pRPA314 or pLJC2 does not affect the level of transcription of *bfpD*. Furthermore, pLJC1 recovered from cells containing pLJC1 and either pRPA314 or pLJC2 is able to direct expression of BfpD when electroporated into TOP10F' with pTrcphoA, indicating that pLJC1 remains intact. Interestingly, BfpD was detectable in TOP10F' containing pLJC1, pRPA314 and pLJC2. Thus, we hypothesized that BfpD interacts with BfpC or BfpE and is degraded in TOP10F' containing pLJC1 and pRPA314 or pLJC2. However, in cells harboring pLJC1, pRPA314 and pLJC2, BfpD, BfpC and BfpE interact to form a stable complex. Consistent with our hypothesis, in cell localization experiments BfpD was found to localize both to the cytoplasm-containing soluble fraction and to the inner membrane-containing soluble fraction prepared from TOP10F' harboring pLJC1, pRPA314 and pLJC2. In contrast, BfpD was only found in the soluble fraction of cells harboring pLJC1 alone.

Finally, using a GAL4-based yeast two-hybrid system, we confirmed the following interactions: the cytoplasmic N-terminus of BfpC and BfpD; the N-terminus of BfpC and the cytoplasmic N-terminus of BfpE; BfpD and the N-terminus of BfpE; the N-termini of BfpC and BfpE; and a small cytoplasmic loop of BfpE and BfpF. In addition, we demonstrated that BfpD, the N-terminus of BfpE and BfpF each interact with themselves.

In conclusion, we have shown interactions between several cytoplasmic and cytoplasmic membrane components of the Bfp biogenesis machine that may be required for formation and/or stabilization of this putative assembly complex.

CHARACTERIZATION OF THE TYPE II SECRETION COMPONENT EPSE

Jodi Camberg[1] and Maria Sandkvist[1,2]
[1]Institute for Biomedical Sciences, George Washington University, Washington, DC 20037
[2]Jerome H. Holland Laboratory, Department of Biochemistry, American Red Cross, Rockville, Maryland 20855

The type II secretion pathway is responsible for translocating proteins across the outer membrane in Gram-negative bacteria. In *Vibrio cholerae*, the type II pathway is comprised of 15 gene products denoted Eps proteins that mediate extracellular secretion of cholera toxin. Previous fractionation data have indicated that the type II component EpsE associates with the cytoplasmic membrane through an interaction with the inner membrane component EpsL, and this interaction is stabilized by another type II inner membrane protein, EpsM. Deletion of EpsL renders EpsE cytoplasmic. This EpsL-dependent membrane association/dissociation of EpsE may suggest the presence of a cycling mechanism for EpsE through the formation of an EpsE/L/M trimolecular complex. Our data utilizing surface plasmon resonance indicate that purified EpsE$(His)_6$ can bind an EpsL/M$(His)_6$ complex in solution, further supporting the existence of an EpsE/L/M complex. Additionally, EpsE contains an ATP-binding motif suggesting that EpsE may be a kinase or an ATPase. To explore the enzymatic activity of EpsE, we examined whether EpsE is capable of ATP hydrolysis. In this study, we report that recombinantly purified EpsE is capable of hydrolyzing ATP *in vitro*, and the addition of EDTA to the reaction abolished this activity. A point mutation in the ATP-binding motif of EpsE reduced its ATP hydrolyzing activity. EpsE may supply the envelope-spanning type II apparatus with energy to facilitate outer membrane translocation by stimulating apparatus assembly or transducing energy through other components including EpsL and EpsM.

THE ROLE OF EPSM IN POLAR ASSEMBLY OF THE TYPE II SECRETION APPARATUS OF *V. CHOLERAE*

Maria E. Scott* and Maria Sandkvist
Dept. of Biochemistry, American Red Cross, Jerome H. Holland Laboratory, 15601 Crabbs Branch Way, Rockville, MD 20855

Vibrio cholerae is a human pathogen responsible for thousands of deaths per year. The major symptoms of this infection are caused by cholera toxin. Secretion of cholera toxin and several other proteins that include hemagglutinin/protease (HAP) is mediated by the type II secretion apparatus encoded by the *eps* operon. A green fluorescent protein fusion (GFP) to EpsM was prepared and shown to rescue the secretion defect in a *V. cholerae epsM* mutant. Fluorescence microscopy in living cells of the *V. cholerae epsM* mutant that expressed GFP-EpsM revealed that the location of the Eps apparatus was confined to the old pole. Furthermore, studies in single cells with fluorescent casein combined with GFP-EpsM showed that extracellular Eps-dependent HAP secretion occurred at the pole of the cell and colocalized with GFP tagged EpsM. This suggests that polar localization of the Eps complex may be required for directed secretion of virulence factors.

The Eps components are believed to work as a coordinated group and to form a multiprotein complex to support protein secretion. Previous investigations show that EpsE, EpsL, and EpsM interact with each other to form a trimolecular complex within the inner membrane. Moreover, EpsE association with the inner membrane is through interaction with EpsL and binding of EpsL and EpsM to each other provide mutual stability. In these studies, experiments were designed to investigate the mechanism of polar localization of the Eps apparatus. While GFP-EpsL and GFP-EpsE could also localize to the pole of *V. cholerae*, they were not confined to the poles of *E. coli*. Instead, GFP-EpsL accumulated uniformly in the membrane and GFP-EpsE was distributed in the cytoplasmic compartment. In contrast, GFP tagged EpsM was retained at the pole in *E. coli* cells, suggesting that EpsM contains all the information in its structure necessary to restrict its location to the pole. Therefore, EpsM could be the protein responsible for localization of all or a subset of Eps components to the pole. Evidence for this, is that localization of GFP-EpsL to the pole in *E. coli* requires the presence of EpsM. Also, coexpression of GFP-EpsE in the presence of EpsL resulted in mostly lateral membrane staining. However, when EpsM was coexpressed with GFP tagged EpsE and native EpsL fluorescence microscopy revealed that GFP-EpsE was redistributed to the cell pole. Taken together the data suggests that EpsM, EpsL, and EpsE form a trimolecular complex that localizes to the pole when expressed in *E. coli* and that EpsM is essential for polar localization of the three proteins. Future studies are planned to address specific mechanisms involved in polar retention of EpsM at the cell pole.

BRUCELLA ABORTUS MUTANTS WITH GROWTH DEFECTS UNDER STARVATION CONDITIONS ARE ATTENUATED IN CULTURED MACROPHAGES AND IN EXPERIMENTALLY-INFECTED MICE.

R. Alcantara*, and R.M. Roop, II.
Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, North Carolina 27858-4354

Brucella abortus is one of the causative agents of human and animal brucellosis. The organism can establish chronic infection by surviving and replicating in macrophages, despite the limited availability of nutrients in the phagosomal compartment, indicating that *B. abortus* can support its nutritional requirements within the host cell. Our purpose, then, was to identify the genes involved in these metabolic activities. Transposon mutagenesis of *B. abortus* 2308 was performed using the suicide plasmid pUT/Km1. Mutants deficient in growth under starvation conditions were identified by comparing growth on enriched and minimal media, and assayed for virulence in vitro and in vivo. After 10 days of incubation at 37°C, 5% CO_2, all mutants and the wild-type parent demonstrated similar growth on the enriched medium. However, several mutants displayed inhibited growth compared to *B. abortus* 2308 on the minimal medium. Some of these mutants exhibited decreased intracellular survival in cultured macrophages and attenuation in infected mice, while others displayed wild-type virulence. Sequence analysis revealed transposon disruptions within *Brucella* genes required for both amino acid and purine biosynthesis. Further characterization of the disrupted loci in the attenuated mutants will provide insight into the nutritional needs of *B. abortus* during intracellular residence in the host macrophage.

PRODUCTION AND TOXICITY OF BACTERIAL VESICLES

Meta J. Kuehn, Dept of Biochemistry, and Molecular Genetics and Microbiology
Duke University Medical Center, Durham, NC 27710

All gram-negative bacteria observed to date, including enterotoxigenic *Escherichia coli* (ETEC), produce outer membrane vesicles. Bacterial vesicles are spherical, approximately 100-200 nm in diameter, and composed of outer membrane proteins, lipids and enclosed periplasm. These vesicles are predominantly released during log-phase growth and production continues throughout stationary-phase. Heat-labile enterotoxin (LT) is a virulence factor responsible for the virulence of ETEC, an important pathogen responsible for traveler's diarrhea. We have found that active LT is secreted by ETEC in association with vesicles and that vesicles are the only means by which LT is transported to the supernatant. Like the homologous *V. cholerae* toxin, CT, LT mediates binding to the host epithelial receptor, GM1. ETEC vesicles bind to host cells in a manner that is GM1-dependent and they are internalized via a caveolae-mediated endocytic process. We demonstrated that LT is secreted by the general secretory pathway (GSP) and binds to LPS on the surface of the cell and vesicle in a site distinct from its GM1 binding site. We examined more closely the association between LT and LPS and found that both CT and LT bind to *E. coli* LPS, but not *Vibrio* LPS, and that this binding requires only the Kdo sugars in the inner core. Despite the equivalent activity CT and LT exhibit in bioassays, disease caused by ETEC is much less severe than that caused by *V. cholerae.* This suggests that the difference between *V. cholerae* and ETEC virulence may depend on the efficiency of toxin secretion and the delivery mechanism. We propose that the LPS and the respective toxin's ability to bind to that LPS is an important factor that influences the severity of ETEC and cholera infections.

Wednesday, February 5
Morning Session

TRANSPOSON MUTAGENESIS OF *BACILLUS ANTHRACIS*: IDENTIFICATION OF MACROPHAGE INVASION LOCI

A. K. Pickering, G. M. Lee and T. J. Merkel
Laboratory of Respiratory and Special Pathogens, CBER, FDA
Bethesda, MD.

Intracellular survival and escape from macrophages is thought to be an important characteristic of *Bacillus anthracis* that contributes to its ability to progress from a local to a lethal systemic infection. Using transposon mutagenesis, we created a pool of *B. anthracis* mutants that were screened using two assays designed to target attenuations in the ability to resist bactericidal activity and more specifically, the macrophage oxidative burst. Seven mutants were selected for further characterization and the chromosomal transposon insertion site was identified in each case by plasmid rescue. Mutants typically exhibited a retarded intracellular growth pattern that was accompanied by an attenuated ability to 'escape' from macrophages *in vitro*. The study of these mutants may provide valuable insights into the earliest events of anthrax infection.

GENETIC ANALYSIS OF THE ROLE OF THE *BRUCELLA ABORTUS* ALKYL HYDROPEROXIDE REDUCTASE, AHPC, IN RESISTANCE TO HYDROGEN PEROXIDE AND VIRULENCE

M. W. Valderas, R. B. Alcantara, J. E. Baumgartner, J. M. Gee, and R. M. Roop II
East Carolina University School of Medicine, Department of Microbiology and Immunology,
Greenville, NC 27834

The chronic nature of infection is the hallmark of brucellosis and is due to the ability of *Brucella abortus* to survive and persist in host macrophages. Macrophage colonization is essential for virulence, but in order to establish infection the brucellae must overcome the effects of exposure to the reactive oxygen intermediates, nutrient deprivation, and acidic pH found in the phagosome. Mutation of the *B. abortus hfq* gene results in significant sensitivity to hydrogen peroxide and acidic pH in stationary phase. This mutant failed to replicate in cultured murine macrophages and demonstrated clearance from infected mice under conditions where the parent established chronic infection. Two dimensional gel analysis of the *B. abortus hfq* mutant identified more than forty genes that require the *hfq* gene product host factor I (HF-I) for optimal expression in stationary phase. One of the gene products identified in this manner is a homologue of the *E. coli* alkyl hydroperoxide reductase AhpC, which is a peroxidase responsible for scavenging low levels of endogenous or exogenous hydrogen peroxide. *B. abortus* has a single periplasmic catalase, and mutation of this gene does not result in an aerobic growth defect, inability to replicate in host macrophages, or in attenuation in mice. It seems reasonable to hypothesize that the *B. abortus* AhpC may function in a manner similar to that of the *E. coli* AhpC, allowing the catalase mutant to survive the oxidative burst of the macrophage by virtue of its peroxidase activity. Consequently, an isogenic *ahpC* mutant designated MWV8 has been constructed from virulent *B. abortus* 2308 by allelic replacement. *In vitro* assays have shown that MWV8 is sensitive to hydrogen peroxide and is unable to replicate in resident murine macrophages, indicating that the role of AhpC may actually be to provide the major hydrogen peroxide scavenging activity of *B. abortus.* Additional *in vitro* and *in vivo* experiments are in progress to determine the contribution of the *B. abortus* AhpC to successful adaptation to the organism's niche.

PSEUDOMONAS AERUGINOSA OUTER MEMBRANE VESICLES PRODUCED DURING EXPONENTIAL GROWTH ASSOCIATE WITH AND ACTIVATE LUNG EPITHELIAL CELLS

Susanne J. Bauman*, Saùl Villalobos and Meta J. Kuehn
Duke University Medical Center, Department of Biochemistry, Durham, NC 27710

Pseudomonas aeruginosa is a gram negative, opportunistic pathogen that is a major cause for morbidity and mortality in individuals with compromised lung function such as in patients with cystic fibrosis (CF). One major cause of lung injury results from the acute inflammatory response to the infection. Outer membrane vesicles are secreted by *P. aeruginosa* as well as many other well-characterized pathogens. To characterize vesicles produced by *P. aeruginosa* and to investigate their interactions with host cells and the immune response, a vesicle purification protocol was developed. Vesicles were purified from lab strain PAO1, an environmental isolate, and two CF isolates. *P. aeruginosa* vesicles were produced during early stationary phase, and appeared to be consumed or degraded during stationary phase. Quantitation of PAO1 vesicles in a culture supernatant revealed that 1-2 % of outer membrane proteins were in vesicles at the end of the vesicle production phase. Purified vesicles were compared by electron microscopy and protein composition. Vesicles from CF isolates were smaller and more regularly spherical than PAO1 or soil vesicles. The protein composition of the vesicles closely resembled that of the outer membrane. Vesicles were comprised of outer membrane porins and CF vesicles were enriched in PaAP aminopeptidase. Since vesicles are likely to come into contact with host cells during an infection, the association of fluorescently labeled vesicles with host cells was examined by confocal microscopy. Vesicles from all four strains associated with A549 human lung epithelial cells and primary human bronchial lavage cells. Quantitation of cell-associated fluorescence revealed that significantly more vesicles from CF strains associated with the lung cells compared to vesicles from PAO1 and soil strains. Vesicles from all strains produced an IL-8 response in both cell types. Taken together, these results suggest that *P. aeruginosa* infecting the CF lung produce vesicles that associate with lung cells and that vesicles probably contribute to the inflammatory response.

AN EVALUATION OF THE ROLE OF THE PERIPLASMIC SUPEROXIDE DISMUTASE IN THE PATHOGENESIS OF *BRUCELLA ABORTUS.*

J. Gee,*[1,2], and R.M. Roop II[1].

[1]Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, NC 27858-4354. [2]Department of Microbiolgy and Immunology, Louisiana State University Health Sciences Center, Shreveport, LA 71130.

The genus *Brucella* is composed of facultative intracellular bacteria that produce chronic infections in human and animal species. Since the brucellae infect and multiply within professional phagocytes such as macrophages, there is a strong correlation between their capacity to survive and replicate within these cells and their ability to establish and maintain chronic infection in the host. Within the macrophage environment, the brucellae are subjected to exposure to numerous environmental stresses, including acidic pH, reactive oxygen intermediates (ROIs), and nutritional deprivation. Therefore, to successfully establish and maintain a chronic infection, the brucellae must adapt to this harsh environment.

It has been shown *in vitro* that the brucellae experience an initial period of killing upon infection of macrophages, but thereafter the surviving intracellular bacteria multiply. This initial period of control of the brucellae by the macrophage is dependent upon exposure of the brucellae to reactive oxygen intermediates (ROIs), such as the superoxide anion (O_2 -) and hydrogen peroxide (H_2O_2). Remarkably, although opsonization of the brucellae with specific IgG or activation of macrophages with IFN-γ increase macrophage production of ROIs, virulent strains of *Brucella* can still resist killing by these cells and demonstrate intracellular replication. Recent genetic and biochemical studies have identified several cellular components that contribute to the resistance of the brucellae to oxidative killing by professional phagocytes. These components include primary antioxidants such as catalase and two forms of superoxide dismutase (SOD): a periplasmic Cu/Zn cofactored SOD (SodC), and a cytoplasmic Mn SOD.

Due to the periplasmic location of SodC and the relative inability of superoxide to cross the inner membrane of gram-negative bacteria, *sodC* homologs in intracellular pathogens have been postulated to detoxify superoxide encountered outside the bacterial cell. Therefore, SodC is thought to provide protection against the oxidative burst of professional phagocytes. In this study we evaluated this possibility by examining the *in vitro* sensitivities of a *B. abortus sodC* mutant to various forms of oxidative stress, as well as its ability to survive and replicate within macrophages *in vitro*. We also evaluated the capacity of the *sodC* mutant to establish and maintain chronic infection in the experimental murine model. Unlike *sodC* deficient strains from other bacteria, the *B. abortus sodC* mutant also displayed significant sensitivity to endogenously generated metabolic superoxide. Using 2D gel electrophoretic analysis, we have found maximal SodC synthesis to occur during the stationary phase of growth, and this increase can be attributed to the presence of the HF-I (Host Factor-I), an important regulator of stationary phase-dependent gene expression. Collectively, these observations raise the possibility that the protection afforded the brucellae by SodC against the exogenous superoxide exposure of the respiratory burst may not be its only function.

A GONOCOCCAL EFFLUX PUMP SYSTEM ENHANCES BACTERIAL SURVIVAL IN AN ANIMAL MODEL OF GENITAL TRACT INFECTION

[1]Ann E. Jerse, [1]Nirmala Sharma, [1]Amy N. Simms, [2]Lori A. Snyder, and [2,3]William M. Shafer. [1]Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD; [2]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA; and [3]Medical Research Service, VA Medical Center, Decater, GA

Active efflux of antimicrobial substances is likely to be an important bacterial defense against inhibitory factors inherent to different host body sites. Two multidrug resistance efflux systems (MtrCDE, FarAB,MtrE) have been identified in *Neisseria gonorrhoeae*, a pathogen of the human genital mucosa. The role of the MtrCDE and FarAB,MtrE efflux systems in antibiotic resistance has been the focus of much research. The selective forces that led to the evolution of these systems in *N. gonorrhoeae*, however, surely pre-date antimicrobial therapy. The MtrCDE efflux system confers high level resistance to bile salts, fatty acids and antibacterial peptides such as protegrins. Efflux of long chain fatty acids occurs via the gonococcal FarAB,MtrE efflux system. The role of multidrug resistance pumps in protecting the gonococcus from these host factors thus far has only been speculated based on in vitro studies and the isolation of efflux pump over-producers from rectal infections, where concentrations of inhibitory substrates are high.

To directly assess the role of active efflux pumps in adaptation of *N. gonorrhoeae* to the genital tract, we tested genetically defined efflux pump mutants of *N. gonorrhoeae* in a female mouse model of gonococcal genital tract infection. The recovery of *mtrD* and *mtrE* mutants, but not a *farB* mutant was reduced from the lower genital tract of mice inoculated with suspensions containing wild type or mutant gonococci. MtrCDE-deficient gonococci, but not the FarAB-deficient mutant, also showed a survival disadvantage compared to wild type gonococci in competitive infection experiments. To identify hydrophobic substrates of the MtrCDE pump that may be present in the genital tract, we tested the sensitivity of the efflux pump mutants to gonadal steroids. None of the mutants were sensitive to 17-β estradiol, however the *mtr* mutants were sensitive to progesterone in vitro. Competitive infection experiments using ovariectomized versus intact mice showed that MtrCDE-deficient gonococci were more rapidly cleared from mice that were capable of secreting gonadal hormones. This result is consistent with the hypothesis that the MtrCDE pump protects *N. gonorrhoeae* from progesterone in vivo, however we cannot rule out the possibility that downstream effects of gonadal steroids may be responsible for the faster clearance of MtrCDE mutants from intact mice.

In summary, we have shown that a multidrug efflux system promotes survival of a bacterial pathogen in the female genital tract. Although multiple inhibitory factors appear to be present in the genital tract, the evolution of the MtrCDE pump may in part have been driven by the need to protect *N. gonorrhoeae* against gonadal steroids and/or their downstream effects.

EXPERIMENTAL HUMAN INFECTION WITH *NEISSERIA GONORRHOEAE*: ROLE OF PILI AND OPACITY PROTEINS IN INFECTION OF THE MALE URETHRA.

Terri S. Hamrick[1], J.A. Dempsey[1], N. Guyer[1], M. Binnicker[3], D. J. Hobbs[1], D. M. Johnston[1], M. M. Hobbs[1,2], M. A. Apicella[3], M. S. Cohen[1,2], and J.G. Cannon[1*]. Departments of Microbiology and Immunology[1] and of Medicine[2], University of North Carolina, Chapel Hill, NC; Department of Microbiology and Immunology, University of Iowa, Iowa City, IA[3]

Two well-characterized adhesins of the gonococcus (GC) are pili and Opacity (Opa) proteins, both of which demonstrate high-frequency antigenic variation. GC isolated from men with gonorrhea are invariably piliated and Opa-positive, which has led to a belief that these components are essential for infection of the male urethra. Studies of GC pathogenesis have been hampered by it's extreme human-adaptation. However, experimental infection of human male volunteers is safe, and reproduces the clinical features of natural gonorrhea. Using the human challenge model, we examined the role of pilin and Opa proteins in infection.

Population dynamics of infection. Tracking the variation of Opa and pilin expression during infection of volunteers with a wild type strain revealed that the phenotype of the GC changed multiple times between inoculation and the appearance of a urethral discharge. The inoculum variant disappeared rapidly, and was replaced by a series of new variants expressing different surface antigens. We believe these changes reflect the sequential outgrowth and disappearance of new clonal populations that can proliferate if they express advantageous pilin and/or Opa phenotypes. Selection of these "waves" of phenotypic variants was not due to antibody responses, but we do not know the identity of the selective pressures.

Infectivity of pilin- and Opa-negative mutants. We constructed two mutant strains: one unable to express pilin and one with all eleven *opa* loci inactivated. Both were predicted to be non-infectious, but gave surprising results in human challenge experiments. The pilin-negative mutant colonized 7 of 8 inoculated subjects, causing urethritis in only 2 of them (inoculum equivalent to wild type ID_{90}). An explanation for the relatively asymptomatic infection caused by this mutant could be that it was less invasive than the parent strain. To test this possibility, we infected primary human male urethral epithelial cells with the pilin-negative mutant *in vitro*. Fewer organisms of the mutant attached to the cells, compared to the wild type. However, the pilin-negative GC that did attach invaded the epithelial cells as well as the wild type, suggesting that the less inflammatory infection caused by the mutant was not due to qualitative differences in GC:cell interactions. The Opa-negative mutant was nearly as infectious as the wild type *in vivo* (4 of 8 subjects infected), and attached to and invaded epithelial cells as well as the wild type *in vitro*. The dispensability of Opa proteins for infection is seemingly inconsistent with the strong selection for Opa expression in the male urethra. The results emphasize that GC virulence is multifactorial and that critical functions promoted by pili and Opa proteins, including attachment, must be redundant.

WINE INOCULATED WITH *HAEMOPHILUS DUCREYI* DEVELOP BACTERICIDAL ANTIBODIES TO NOVEL COLLAGEN BINDING OUTER MEMBRANE PROTEIN DSRB.

Leah E. Cole (1), Kristen L. Toffer (1) (2), Robert A. Fulcher (1), Paul Orndorff (3), and Thomas H. Kawula (1).
(1) University of North Carolina at Chapel Hill, Chapel Hill, NC.
(2) Present address: Groton Laboratories, Pfizer Inc., Groton CT.
(3) NC State University, College of Veterinary Medicine Raleigh, NC .

Chancroid is a sexually transmitted genital ulcer disease caused by the bacterium *Haemophilus ducreyi*. *H. ducreyi* infection does not typically result in the development of protective immunity. Using a swine model of chancroid, we sought to determine if repeated exposure to *H. ducreyi* could promote a protective immune response. Pigs were inoculated with *H. ducreyi* three times over a 28-day period and assessed for organism recovery from lesions two and seven days after each inoculation. Two days after the first and second inoculations, 20 out of 20 biopsies (100%) were culture positive whereas only 11 of 20 (55%) were positive following the third inoculation. Seven days after the first inoculation 17 out of 19 (89%) biopsies were culture positive while only 3 out of 19 (16%) were culture positive after the third inoculation. The number of bacteria recovered from the day seven lesions decreased 100 fold between the first and third inoculation. Thus repeated exposure resulted in a modest level of protection against *H. ducreyi*.

Serum taken from swine shortly after third inoculation killed 75.11% of the bacteria present in a bactericidal killing assay. To determine if protective immunity was humorally based we passively transferred bactericidal swine serum into uninfected pigs and challenged those pigs with *H. ducreyi*. Significantly fewer viable bacteria were recovered from animals that had been infused with bactericidal serum as compared to animals that were infused with normal pig serum or saline solution. Thus, a robust humoral response to *H. ducreyi* may provide protection against infection with this organism.

We identified the majority of targets recognized by antibodies present in the immune pig serum. One of these targets was a previously uncharacterized outermembrane protein that we termed DsrB. Using a *dsrB* mutant strain, we found that this outer protein was a target for bactericidal antibodies, functioned as a collagen binding protein, and is required for full virulence in the swine model of chancroid.

UNDERSTANDING THE ROLE OF MULTIPLE FIMBRIAL ADHESINS IN URINARY TRACT INFECTIONS

Eric L. Buckles* and Michael S. Donnenberg
Division of Infectious Diseases, Department of Medicine
University of Maryland, Baltimore, MD 21201

Fimbriae-mediated adherence is a crucial step in urinary tract pathogenesis by *E. coli*, the most common cause of urinary tract infection. Completion of the genome sequence of uropathogenic *E. coli* strain CFT073, isolated from the blood of a woman with acute pyelonephritis, revealed the presence of genes encoding twelve distinct putative fimbriae, ten fimbriae of the chaperone-usher family and two type IV pili. Fimbriae of the chaperone-usher family include the type 1 (*fim*), two copies of the P (*pap*) fimbriae, as well as the F1C (*foc*), which have been epidemiologically linked to UTIs. In addition, genes related to the Stf pilus of *Salmonella* and MRP pilus of *Proteus mirabilis* (*stf*), Yad (*yad*) fimbriae, genes encoding an unusual putative lipoprotein fimbrial adhesin (*ag*), genes that are type-1 fimbrial related (*ag*), a novel operon with two chaperones (*auf*), and a minimal fimbrial operon (*aa*) are among the chaperone-usher family. Genes for the type IV pili include *ppdDhofBC* and a homologue of a type IV pilus tip adhesin (*ak*). In addition, strain CFT073 has genes encoding curli (*csg*). Previous studies have shown that mutations in the *fim* or *pap* operons of CFT073 did not completely abolish the ability of CFT073 to colonize the mouse urinary tract; therefore, other putative fimbrial operons may compensate for their loss. It is our contention that simultaneous deletion of several fimbrial adhesins would reduce CFT073 colonization and virulence to a greater degree than mutations in individual fimbrial operons. As a prelude to determine which fimbrial operons are important for CFT073 colonization of the urinary tract, we first examined the transcription of the twelve distinct putative fimbriae of CFT073 by reverse transcription-PCR with gene-specific oligonucleotide primers to the major subunits of the fimbrial operons. For *in vitro* analysis, RNA was isolated from bacteria of CFT073 grown to exponential or stationary phase in LB broth. It was demonstrated that the major subunit of all of these operons, with the exception of *aaA*, were expressed during early exponential growth phase in LB broth; however, only *focA, anA, agA, papA, stfA,* and *fimA* expression was detected during the stationary growth phase. These results indicate that CFT073 is capable of expressing at least eleven different fimbrial genes. To determine which genes are expressed *in vivo*, mice were infected with 10^8 cfu from stationary cultures of CFT073 and urine samples were collected at 4, 24, and 48 hours. Urine samples were then combined, bacteria isolated, and RT-PCR was performed. Out of four or five valid experiments for each operon at the indicated time period, we were able to detect expression of each fimbrial operon except for *aa* and *ppd,* at least one time, indicating that these operons are expressed in the mice urinary tract and may play a role in CFT073 colonization. Interestingly, *focA* was the only major subunit expressed at all time periods under the conditions tested. Currently, we are constructing mutants and exploring the role of F1C fimbriae in CFT073 colonization of the mouse urinary tract.

GENOME HETEROGENEITY IN A BACTERIOPHAGE ISOLATED FROM *BORDETELLA AVIUM.*

Holly Kuzmiak, Sara Shanda, Octawia Wozniak, Duncan Maskell, and Louise Temple.

A temperate, transducing bacteriophage (Ba1) infecting *Bordetella avium*, a pathogen of birds, was isolated from strain Wampler by passage of culture supernatant through the sensitive *B. avium* strain, 197N. DNA from a gradient purified preparation of Ba1 was sequenced and found to contain two apparently complete genomes of 43,398 and 38,526 bases (Ba1-1 and Ba1-2, respectively). The larger species was much more highly represented in the sequenced clones. Approximately 2/3 of the genomes are >80% identical at the DNA level; these regions encode putative structural proteins for the phage particles, which possess icosahedral heads, contractile tails, and tail fibers. The remaining divergent regions of the genomes encode putative regulators, such as recombinase and repressor proteins. Seven other *B. avium* strains isolated from sick or healthy birds have been shown to produce phage that infect strain 197N. PCR and Southern blotting analyses using genomic DNA of lysogens has revealed that 4 of 8 lysogens contain both Ba1-1 and Ba1-2 sequences; the remaining 4 contain only Ba1-1 sequences. In contrast, all phage DNA preparations contain both Ba1-1 and Ba1-2 sequences after passage through 197N. Ba1-2 sequences are also present in genomic DNA from the sensitive strain, 197N. Further PCR using primers directed outwards from ends of the chromosomal sequences showed that for the Ba1-1 sequence, the ends are adjacent in the chromosome of the lysogen, Wampler, whereas the ends of Ba1-2 are not adjacent in 197N. We hypothesize, based on these observations, that Ba1-2 exists in 197N (and other *B. avium* strains) as a defective phage that can be induced by helper phage (Ba1) infection or that the chromosomal sequences defined as Ba1-2 are a collection of recombined fragments resulting from the infection by competent phage, likely represented as the sequence we designate Ba1-1. Work is ongoing to determine which, if either, of these hypotheses is correct.

WHAT SHOULD BE THE BASIS FOR AN EFFICACIOUS VACCINE TO PROTECT AGAINST *HAEMOPHILUS DUCREYI* INFECTION?

Tom Kawula

Haemophilus ducreyi is the etiologic agent of chancroid, a sexually transmitted genital ulcer disease. Numerous studies suggest that natural and experimental infection with *H. ducreyi* does not elicit a protective immune response. We have used a swine model of chancroid to investigate both the type and targets of an immune response that could protect against subsequent *H. ducreyi* infection. We present evidence that infected animals produce a modest antibody response to a very limited number of *H. ducreyi* antigens following repeated inoculations with this organism. The appearance of bactericidal antibodies coincided with enhanced protection against *H. ducreyi* infection. Transfer of these sera to naïve animals provided similar protection against infection. Three potential outermembrane targets of this antibody response have been identified, hemolysin, D-15, and a previously undescribed protein termed NcaA that confers bacterial attachment to collagen. We conclude that a robust humoral immune response to *H. ducreyi* could provide immunity to infection with this organism.

Poster Session

POSTER # 1

EXPRESSING *BACILLUS ANTHRACIS* PROTECTIVE ANTIGEN IN *ESCHERICHIA COLI* AND *BRUCELLA MELITENSIS.*

A.B. Bandara, V. Dobrean, S. Poff, N. Sriranganathan, G.G. Schurig and S.M. Boyle.
Department of Biomedical Sciences & Pathobiology, Virginia Polytechnic Institute & State University, Blacksburg, Virginia.

The objective of this work is to develop a Brucella-Anthrax vaccine for human. Attenuated *Brucella melitensis* vaccine candidate WR201 (Δ*purEK* mutant) developed by Walter Reed Army Institute of Research is used in this work. The 2.3-kb *pag* gene, which encodes *Bacillus anthracis* protective antigen (PA) was cloned downstream from the GroE promoter in plasmid pBBGroE to produce pBBGroE/PA. From this plasmid, the GroE/PA cartridge was isolated and subcloned into pBBR4MCS to produce pBB4PA. Strain WR201 was electroporated with plasmid pBB4PA to produce strain WR201PA. Western analyses using rabbit anti-PA polyclonal serum indicated that *Escherichia coli* carrying pBB4PA, and *B. melitensis* strain WR201PA each produced an approximately 65-kDa protein product, which was equivalent in size to the full length PA. In addition, this serum reacted with a few more protein products between 4 to 30-kDa in size, which may have arise due to degradation of PA. The strain WR201PA is being tested for its level of attenuation and protective efficacies against virulent strains of *B. melitensis* and *B. anthracis.*

POSTER # 2

TRAFFICKING AND RESIDENCE OF *BRUCELLA ABORTUS* WITHIN HUMAN MONOCYTES

Bryan H. Bellaire[1], R. Martin Roop II [2]and Jim Cardelli[1].

[1]Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, Louisiana, USA 71130-3932; [2]Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, North Carolina, USA 27858-4354

Survival and replication of *Brucella abortus* within host macrophages is critical for virulence of this bacterial pathogen. To characterize the manner by which internalized *B. abortus* were processed, human THP1 monocytic cells were infected with opsonized *B. abortus* 2308 and the vesicles containing bacteria were characterized regarding the presence or absence of cellular markers detected by immunofluorescence microscopy. Rapidly following uptake Brucellae containing vesicles acquired early endosomal (EEA1) and late endosomal/lysosomal (Lamp1) markers. EEA1 retention was transient in nature as brucellae containing vesicles lost EEA1 by 2 hours while levels of Lamp1 co-localization remained high throughout (96 hours p.i.). In contrast, these vesicles did not appear to contain detectable mannose-6-phosphate receptor (late endosomal marker) or Cathepsin D (late endosomal/lysosomal marker), even up to 48 hours p.i. suggesting that *B. abortus* containing vesicles do not fuse with lysosomes to generate phago-lysosomes. In addition, these late phagosomes (post 6 hours) were acidic but did not appear to interact with other phagosomes or with endosomes. Interactions with *B. abortus* containing vesicles were compared to results obtained with *E. coli*, latex bead particles and heat killed brucellae. These results suggest that the trafficking of *B. abortus* within human monocytes initiates through the endosomal pathway but is altered soon after leading to a vesicular compartment that does not interact completely with endosomes or lysosomes.

POSTER # 3

EVALUATION OF *SPY1600*, A PROPOSED HYALURONIDASE GENE OF *STREPTOCOCCUS PYOGENES*.

Karin M. Berling and Wayne L. Hynes
Department of Biological Science, Old Dominion University, Norfolk, VA 23529.

Streptococcus pyogenes can cause a variety of human diseases ranging from asymptomatic to life threatening. How one organism does this remains to be elucidated. One possibility is the involvement of the large number of virulence factors possessed by the organism. The recent sequencing of three streptococcal genomes has indicated the existence of several unknown genes, some of which may encode possible virulence factors. Among these is *Spy1600*, which based on its nucleotide sequence similarities has been proposed to encode a hyaluronidase, a type of enzyme that is capable of breaking down the hyaluronic acid found in host connective tissue. According to the sequences of *Spy1600* there is no signal peptide within the first forty amino acids of the N-terminal, which would suggest an intracellular product or a non-typical secretion mechanism. The aim of this project was to determine whether *Spy1600* is conserved in other strains of *S. pyogenes* and does the gene encode an active hyaluronidase. Primers were designed from a published sequence, and the gene amplified by PCR. The gene was detected in all 50 strains tested, indicating a conserved genetic region in the genome. The *Spy1600* genes from two different strains (M-type 1 and M-type 22) were cloned, transformed into *E. coli,* sequenced, and tested for hyaluronidase activity using a standard hyaluronidase assay. The gene was fused to a His-tag containing leader peptide and expressed using an inducible *E. coli* promoter. Neither of the cloned genes show activity, either intra- or extracellularly. The recombinant protein was also isolated by affinity chromatography and is, as anticipated, approximately 66.5 kDa. The purified protein did not show any hyaluronidase activity when assayed. Therefore it appears that *Spy1600* does not seem to code for the virulence factor hyaluronidase, and the actual function of any protein produced by this gene remains to be established.

POSTER # 4

THE *GADB* AND *GADC* GENE PRODUCTS DO NOT CONTRIBUTE TO ACID RESISTANCE OF *BRUCELLA ABORTUS*.

Tim Brown, Michelle Wright-Valderas, and R. Martin Roop II, Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, NC 27858-4354

Brucella abortus is an extremely successful intracellular pathogen of humans and domestic animals. This bacterium's ability to cause disease is a direct result of its ability to persist within host macrophages. It has been well documented that the brucellae must withstand exposure to a low pH environment within the phagosome in order to establish and maintain the chronic infection state. Experimental evidence has shown that HF-I, the product of the *hfq* gene, is required for the efficient entry of *B. abortus* into stationary phase physiology. This physiologic transition is also essential for the maintenance of chronic infection in experimental and natural hosts. One of the consequences on entry into stationary phase for many bacteria is an enhanced resistance to acidic pH, and it is notable that the *B. abortus hfq* mutant Hfq3 displays a pronounced sensitivity to low pH in vitro that is only observed during stationary phase. In *Escherichia coli*, HF-I is an RNA binding protein that is required for translation of the mRNA encoding the stationary phase specific alternate σ factor RpoS. Although the presence of a functional *B. abortus* RpoS homolog has yet to be experimentally established, 2D gel analysis has shown that > 40 *B. abortus* genes require HF-I for their optimal expression during stationary phase. One of these genes is *hdeA*, which encodes a small chaperone that has been shown to be important in acid resistance in the enterics. Immediately upstream of *B. abortus hdeA* is an operon consisting of three genes, *gadB, gadC,* and *gls*, encoding glutamate decarboxylase, a putative γ-amino-butyric acid/glutamate antiporter, and glutaminase, respectively. The predicted products of these genes share significant amino acid identity with the *Escherichia coli* GadB (72.9% identity), GadC (29.2% identity), and glutaminase (36.5% identity). GadB catalyses the irreversible α-decarboxylation of glutamate to form γ-amino-butyric acid (GABA), this reaction consumes a hydrogen ion and releases CO_2. The GABA produced in this reaction is then pumped out of the cell with the concomitant import of glutamate. In *Escherichia coli*, the *gadBC* genes are RpoS regulated and play an important role in stationary phase resistance to low pH. In light of the homology shown with *E. coli* genes, and the known function of these genes in enteric organisms, it seems reasonable that the *B. abortus gadBCgls* operon products contribute to the survival of *Brucella abortus* in the acidic environment within the macrophage. To determine if *gadB* and *gadC* contribute to stationary phase acid resistance in *B.* abortus and virulence in the host, the isogenic *gadB* mutant HB1 and *gadC* mutant HB2 were constructed from virulent *B. abortus* 2308 by gene replacement. The *in vitro* and *in vivo* phenotypes of the *B. abortus gadB* and *gadC* mutants were compared to those of 2308, and the gene products did not play a role in either *in vitro* acid resistance, or in long-term survival in the experimental mouse model.

POSTER # 5

INTERRELATIONSHIP OF *P. AERUGINOSA* QUORUM SENSING SYSTEMS

M. Worth Calfee and Everett C. Pesci
Brody School of Medicine at East Carolina University

Pseudomonas aeruginosa is an opportunistic human pathogen that controls multiple virulence factors through intercellular signals in two quorum sensing systems (*las* and *rhl*). Each system is comprised of a transcriptional activator protein, LasR or RhlR, which controls expression of the autoinducer synthase genes, *lasI* or *rhlI*, respectively. LasR and RhlR bind corresponding autoinducers *N*-3-oxododecanoyl homoserine lactone (3-oxo-C_{12}-HSL) or *N*-butyryl homoserine lactone (C_4-HSL), respectively. A third cell to cell signal, PQS (2-heptyl-3-hydroxy-4-quinolone), is intimately involved in the *P. aeruginosa* quorom sensing hierarchy. Intersystem effects are frequently observed in *P. aeruginosa*, yet are poorly characterized. Genes involved in quorum sensing systems are often regulated by an intricate web of activators, repressors, and effectors. Insight into how these systems communicate and affect each other could elucidate mechanisms of virulence. Our data suggest that RhlR is activated by PQS in the absence of both autoinducers (3-oxo-C_{12}-HSL and C_4-HSL). We have also found that RhlR can have a negative effect on virulence factor production under certain conditions. In the presence of PQS, RhlR enhanced *rhlA* and *lasB* transcription, but inhibited pyocyanin production. We also found that LasR could be negatively effected by PQS. Results presented here are an attempt to further the understanding of the quorum sensing hierarchy in *P. aeruginosa*.

POSTER # 6

COMPONENTS OF THE OUTER MEMBRANE SUBASSEMBLY OF THE TYPE IV BUNDLE-FORMING PILUS BIOGENESIS MACHINE OF ENTEROPATHOGENIC *ESCHERICHIA COLI*

Anu Daniel*, Wiebke Schreiber and Michael S. Donnenberg
Division of Infectious Diseases, School of Medicine, University of Maryland.

Enteropathogenic *Escherichia coli* (EPEC), a major cause of infantile diarrhea in developing countries, expresses a type IV fimbria known as the bundle-forming pilus (BFP). BFP is involved in localized adherence of EPEC, which is characterized by formation of defined bacterial clusters on host cells. BFP biogenesis is dependent upon a large EAF (for EPEC adherence factor) plasmid. The EAF plasmid harbors a cluster of 14 *bfp* genes that code for BFP proteins. The first gene of the cluster, *bfpA* encodes prebundlin, the major structural subunit of BFP. Prebundlin is cleaved to its mature form by a prepilin peptidase encoded by *bfpP*. Ten of the remaining genes encode essential components of a putative Bfp biogenesis machine. Several genes of the cluster show similarity to type IV pilus biogenesis genes in other Gram-negative pathogens, however several have no known homologs. The *bfpB* gene encodes an outer-membrane protein belonging to the secretin family. BfpG has been proposed to play a role in BfpB targeting to the outer membrane or in BfpB stability. The *bfpU* gene encodes a protein localized to both the periplasmic space and the cytoplasm whose precise function is not known. We hypothesized that BfpU is a part of the outer membrane complex and interacts with BfpB and/or BfpG. To characterize the pilus sub-machinery at the outer membrane, we investigated protein interactions using chemical cross-linking and pull-down assays. Proteins were cross-linked in EPEC strains carrying either His-tagged BfpU or His-tagged BfpG and the complexes were purified using metal-affinity chromatography. Since the chemical cross-linker can be cleaved by reducing agents, individual proteins in the complexes could be identified following SDS-PAGE in the presence of a reducing agent and western blotting with specific antibodies. Western blot analyses revealed that BfpB was present in cross-linked complexes purified with either BfpU-His or BfpG-His. BfpU was present in BfpG-His purified complex and BfpG was present in BfpU-His purified complex. Neither complex contained the bitopic inner membrane protein BfpC indicating that the purified complexes may represent an outer membrane subassembly of the biogenesis machine. Interestingly, complexes purified using BfpU-His contained bundlin while bundlin was not detected in the complexes purified using BfpG-His, indicating that BfpU may play a specific role in BFP biogenesis that involves direct interactions with bundlin. Additional experiments are being conducted to further characterize the protein complexes at the outer membrane surface.

POSTER # 7

TRANSCRIPTIONAL ANALYSIS OF THE *PSEUDOMONAS AERUGINOSA* PA103 O-ANTIGEN GENE CLUSTER

Antonio DiGiandomenico, Mary Brinig, Jessica Pritchard, Teresa Urban, and Joanna B. Goldberg
Department of Microbiology, University of Virginia, Charlottesville, VA

Pseudomonas aeruginosa is a gram-negative bacterium synominously associated with lung infections in cystic fibrosis (CF) patients. In the early stages of infection, *P. aeruginosa* lipopolysaccharide (LPS) is characterized by a smooth phenotype, containing many O-antigen repeating units. However, in chronically infected individuals, the LPS are observed to have a rough phenotype defined by having few or no O-repeat units. These differences in LPS suggest that O-antigen expression may be regulated by environmental stimuli. Previous work from our laboratory has described the identification, cloning, and sequencing of the genes responsible for the biosynthesis of the *P. aeruginosa* PA103 serotype O11 O-antigen. Reverse transcriptase (RT)-PCR of this locus was performed on PA103 grown at 37 C to mid-log phase; results revealed that each gene is transcribed in the same direction. Notably, we were unable to obtain RT-PCR products from three areas of the locus, suggesting that expression of O-antigen is regulated from separate promoter regions. To identify putative promoters, each region was cloned into the CTX-*lacZ* based reporter plasmid for site-specific integration into the *P. aeruginosa* PA103 genome to monitor gene expression as a function of β-galactosidase activity. Promoter activity of these gene fragments will be discussed. Work has also focused on identifying putative *trans*-acting proteins involved in O-antigen biosynthesis. Raymond *et al.* (J Bacteriol; 2002 184:3614-3622) reported the cloning and sequencing of the O-antigen gene locus from a representative of the twenty *P. aeruginosa* serotypes. Analysis of the DNA sequences upstream of the first recognized O-antigen gene of the locus, *wzz*, revealed a unique gene, *orfA*, predicted to encode a hypothetical protein of approximately 100 amino acids with no known homolog. Because of the close proximity of this gene to those involved in O-antigen biosynthesis, we hypothesized *orfA* may be involved in regulation of O-antigen expression. To test this hypothesis, an *orfA* mutant of PA103 was constructed using an allele replacement strategy in which the *orfA* gene was disrupted by the insertion of a gentamicin resistance (Gm^r) cassette. The phenotype of this mutant, $PA103_{orfA::Gm^r}$ will be discussed.

POSTER # 8

COMPARATIVE IMMUNOGENICITY OF ALPHA AND BETA BUNDLIN PEPTIDES AND PILINS

Paula J. Fernandes and Michael S. Donnenberg
University of Maryland, Baltimore, MD.

Type IV pili are important virulence factors expressed by a number of Gram-negative pathogens. In typical EPEC the EAF plasmid-borne type IV bundle-forming pilus (BFP) is responsible for autoaggregation and localized adherence (LA) phenotypes and required for full virulence. BFP consists of repeating monomers of the structural subunit, bundlin, encoded by the *bfpA* gene. Bundlin is thought to resemble pilins of type IV pili from *Pseudomonas aeruginosa* and *N. gonorrhoeae* for which crystal structures have been determined. The mature pilin proteins consist of a long, hydrophobic amino-terminus believed to be buried within the pilus, involved in pilin-pilin interactions and a globular C-terminal head composed of anti-parallel beta sheets. Previously, sequence analysis of 19 EPEC strains revealed 9 *bfpA* alleles clustering into two major groups (alpha and beta) with the majority of variation, including an excess of non-synonymous substitutions occurring in the 3' two thirds of the gene. Given the proposed tertiary structure of bundlin, the majority of sequence variation may correspond to surface-exposed regions of the native protein thus implicating selective pressures by the host immune system. The proposed protein structure along with variations in amino acid sequence suggests the presence of variable immunodominant epitopes. Such epitopes may contribute to escape from host immune responses. In a preliminary study, a peptide corresponding to the most variable region of the bundlin protein from an α_1 molecule elicited a strong antibody response in rabbits, while the corresponding peptide from a β_5 molecule was poorly immunogenic. To test whether sequence variation affects immunogenicity, two *bfpA* alleles were chosen for further study, α_1 and β_6. The C-terminal end of each allele (encoding residues 25-280 of the mature protein) was fused to the codons for the *dsbA* signal sequence and for six histidine residues to allow the protein to be over-expressed in the periplasm and purified on a nickle column under native conditions. In addition, peptides incorporating residues 137-155 and 136-155 of the mature α_1 and β_6 proteins respectively were used to raise polyclonal antibodies. Proteins and peptides were tested for immunogenicty in mice and for cross-reactivity against the heterologous antigens by ELISA. The α_1 peptide was found to be strongly immunogenic in mice. Conversely, the β_6 peptide showed little immunogenicity (titers <1:10). Anti-_1 peptide antibodies reacted to _1 histidine-tagged protein implying that this region of the native protein is surface-exposed. Anti-_1 peptide antibodies did not cross-react with _6 peptide, most likely due to the lack of a common epitope within this short sequence. Animals immunized with _6 histidine-tagged bundlin cross-reacted strongly against _1 protein, indicating the presence of common epitopes.We have confirmed the hypothesis that a peptide derived from a surface-exposed region of an _ bundlin protein, which is subject to diversifying selection, is more immunogenic than the corresponding peptide from a bundlin molecule. Bundlin sequence variations may have important implications for protective immunity against EPEC expressing homologous and heterologous bundlin proteins and hence for vaccine development.

POSTER # 9

A POTENTIAL ROLE FOR CYTOLETHAL DISTENDING TOXIN IN *HAEMOPHILUS DUCREYI* IMMUNE EVASION AND PATHOGENESIS

Robert A. Fulcher, Kristen L. Toffer, Leah E. Cole, Eric J. Hansen, Thomas H. Kawula

The bacterium *Haemophilus ducreyi* is the causative agent of the genital ulcer disease chancroid. *H. ducreyi* is highly transmissible via sexual intercourse, and individuals with chancroid have a dramatically increased risk of contracting other sexually transmitted pathogens, including HIV. Furthermore, *H. ducreyi* infection fails to provide protective immunity from subsequent exposure to the bacterium. As an obligate human pathogen *H. ducreyi* harbors several genes implicated in pathogenesis, including the *cdtABC* gene cluster encoding cytolethal distending toxin (CDT). CDT is a tripartite toxin expressed by some isolates of Gram-negative bacterial species including *Escherichia coli, Shigella dysenteriae, Campylobacter jejuni,* and *Actinobacillus actinomycetemcomitans*. CdtA and CdtC likely facilitate the delivery of CdtB to the eukaryotic target cell. As the primary cytotoxic factor, CdtB bears homology to the DNase I family, explaining the resemblance of intoxicated cells to those that have sustained double-stranded DNA breakage. Target cells arrest in G1 or G2 of the cell cycle (depending on cell type) and before death take on a distended morphology due to continued cell growth without division. Various cell types are susceptible to CDT intoxication, including cells of the immune system such as Jurkats, human primary T cells, and immature dendritic cells. We employed our juvenile swine model for an *in vivo* assessment of the contribution of CDT to *H. ducreyi* pathogenesis, and found that upon initial inoculation, mutants unable to express CDT produced lesions equivalent to those elicited by the isogenic parental strain. To examine development of the adaptive immune response, we inoculated pigs twice at two-week intervals with either CDT-deficient or wild type *H. ducreyi*. Upon a third challenge using the wild type strain, pigs that had previously received *cdt*$^-$ inoculations showed reduced bacterial burden and histopathology relative to pigs primed with wild type. Accompanying this reduced severity was an increase in host serum bactericidal activity relative to that of serum from pigs with three wild type inoculations. These findings suggest that CDT may be capable of dysregulating the immune response to *H. ducreyi* infection. We are using a murine model to allow us to examine the impact of CDT on the development of cell-mediated and humoral immunity against the surrogate antigen chicken ovalbumin (OVA). This model allows us to express OVA along with any or all of the CDT subunits from plasmid DNA constructs injected into the experimental host. By examining the contributions of the CDT holotoxin and its constituent components within the context of this model and the well-characterized OVA antigen system, we will assess the potential of CDT to deflect or suppress the adaptive immune response to *H. ducreyi*.

POSTER # 10

IDENTIFICATION OF FUNCTIONAL RESIDUES WITHIN THE LASA PROPEPTIDE OF *PSEUDOMONAS AERUGINOSA* THROUGH RANDOM POLYMERASE CHAIN REACTION MUTAGENESIS

Kerian K. Grande and Dennis E. Ohman
Department of Microbiology and Immunology
Medical College of Virginia of Virginia Commonwealth University
Richmond, Virginia

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen and is ubiquitous in nature. *P. aeruginosa* causes infections in immunocompromised patients, including those with cystic fibrosis. It secretes a large number of toxic and degradative enzymes that serve as virulence factors. The LasA protease is a secreted 20-kDa protein with elastin-degrading activity, and it also has staphylolytic activity. LasA is initially synthesized as an inactive preproenzyme that is secreted by a Type II secretion system. ProLasA has a 174 residue NH2-terminal propeptide that is rapidly processed to yield the catalytically active mature form. The propeptide region is required for secretion of LasA. It is hypothesized to act as an intramolecular chaperone that is important for the secretion and proper folding of LasA protease, but the mechanisms are unknown. In order to identify residues important for chaperone activity, random PCR mutagenesis was employed using Taq DNA polymerase. A 490-base pair region of the propeptide was targeted for mutagenesis, and 500 random clones were screened in *P. aeruginosa* for defects in the production of active LasA using agar plates containing heat killed *Staphylococcus aureus*. Clones that produced smaller zones of clearing are being characterized quantitatively for staphylolytic activity, for intracellular accumulation of proLasA and for secretion of LasA by Western blot analysis. So far, several putative mutant clones have been obtained, and the sequence analysis on one of them showed a single amino acid residue was altered. Characterizing mutant *lasA* alleles will reveal propeptide residues important for protein conformation, secretion, and putative points of contact between the propeptide and the mature protease.

POSTER # 11

INTERACTION BETWEEN THE OPACITY (OPA) PROTEINS OF *NEISSERIA GONORRHOEAE* AND CARCINOEMBRYONIC ANTIGEN FAMILY (CEACAM) AND HEPARAN SULFATE PROTEOGLYCAN (HSPG) RECEPTORS: ANALYSIS OF THE RECEPTOR BINDING SITE OF OPA PROTEINS

Nanette B. Guyer, Robin R. Craven, Nathan W. Rigel, Kara L. Pivarski, Janne G. Cannon

Neisseria gonorrhoeae, which causes the sexually transmitted disease gonorrhea, is a pathogen that only infects humans. The Opacity (Opa) proteins of *N. gonorrhoeae* are outer membrane proteins that mediate interaction between the bacterium and the host. A gonococcal strain can express up to 12 distinct Opa protein variants and the sequence of these variants differs from strain to strain. The antigenic variability of Opa proteins is due to the presence of a semi-variable (SV) and two hypervariable (HV1 and HV2) regions that punctuate the conserved portion of Opa protein sequence. Opa proteins are recognized by two classes of eukaryotic cell surface receptors: heparan sulfate proteoglycan (HSPG) and members of the Carcinoembryonic Antigen (CEACAM) family. In strain MS11, Opa proteins with different sequences recognize different subsets of receptors, but the structural basis for this differential recognition is not understood. To investigate the relationship between Opa sequence and receptor recognition we evaluated the receptor binding properties of the Opa proteins of strain FA1090, the only other strain for which the sequence of the entire *opa* gene repertoire is known. We tested a panel of FA1090 variants that singly expressed Opa proteins A-K for binding to heparin, a receptor analog of HSPG, and to recombinant CEACAM 1, 3, 6, 8 and CEA proteins. The receptor binding pattern of FA1090 Opa proteins had similarities to that of MS11: Opa variants displayed differential recognition of HSPG and 4 of 5 CEACAM receptors. In many cases, Opa proteins with identical receptor specificities did not have the same HV1 and HV2 regions, suggesting that receptor recognition is determined by the particular combination of HV sequences. Mutational analysis of recombinant OpaB and OpaI proteins provided further evidence that HV regions contribute to binding specificity. We performed site-directed mutagenesis at each position in HV1 and HV2, substituting the resident amino acid with an alanine residue. Mutations at several positions within HV1 and HV2 resulted in partial or complete loss of CEACAM binding, suggesting that the receptor binding site is comprised of amino acids from both HV regions. Together, these experiments indicate that the receptor specificity of Opa proteins is defined by the HV regions and that binding is dependent on specific amino acids within HV1 and HV2.

POSTER # 12

INFLUENCE OF PREGNANCY ON THE PATHOGENESIS OF LISTERIOSIS IN MICE INOCULATED INTRAGASTRICALLY.

Terri S. Hamrick*[1], John R. Horton[2], Edward A. Havell[2], Ida W. Smoak[2], and Paul E. Orndorff[2]
[1] Department of Pharmaceutical Sciences, Campbell University School of Pharmacy, Buies Creek, NC 27506 [2] Department of Microbiology, Pathology and Parasitology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606

Pregnancy increases the risk of listeriosis, a systemic disease caused by *Listeria monocytogenes*. Despite the well-known association of listeriosis with pregnancy, there is incomplete agreement on the reasons for the increased risk. In our studies, gravid mice were compared to nongravid female mice for changes in disease severity (as measured by lethality) and infectivity (as measured by the level of liver and spleen colonization from 24 through 120 h post inoculation). We employed two listerial strains; one was a serotype 4nonb strain (F6214-1) previously identified as mouse virulent via intragastric (gavage) administration. The other strain was a serotype 1/2a strain (10403S) previously not associated with virulence in mice via gavage. We found that neither strain produced fatalities when gavage administered to nonpregnant mice (dose range of 10^6-10^9 cfu/ mouse). However, in pregnant mice inoculated at 7.5 gestational days, strain F6214-1 (but not 10403S) produced a lethal infection (dose range 10^6-10^8 cfu/mouse). With regard to infectivity, both pregnant and nonpregnant females were less susceptible to infection by strain 10403S compared to F6214-1. If administered via tail vein, the two strains showed similar levels of liver and spleen infectivity, suggesting that 10403S attenuation involved a defect in intestinal escape. However, both strains were equal in binding to, and plaquing upon, cultured mouse enterocytes. The ability of strain F6214-1 to cause lethal disease in pregnant animals was not correlated with an increased incidence or level of liver and spleen colonization over nonpregnant females. Also, pregnant animals were not more susceptible to infection by a listeriolysin O-deficient (*hlyA*::Tn917) insertion mutant (which failed to persist even in the colon of all inoculated mice). Rather, the incidence of lethality in gravid females correlated with infection of concepti (embryos and their surrounding decidual covering). Infected concepti may serve as a source of listeria unique to the gravid female and be ultimately responsible for the increased lethality of the disease in the pregnant animal.

POSTER # 13

EVALUATION OF THE *XTHA* HOMOLOGS OF *BRUCELLA ABORTUS* IN RESPONSE TO OXIDATIVE STRESS

Michael L. Hornback* and R. Martin Roop II
The Brody School of Medicine of East Carolina University

Within host macrophages, *Brucella abortus* resides in phagosomes and must survive exposure to harsh environments including low pH, nutrient deprivation, and exposure to reactive oxygen intermediates (ROIs), including superoxide radicals and hydrogen peroxide. Previous studies have shown that in infected murine peritoneal macrophages, intracellular brucellae are killed over the first 12 to 24 hours. However, after this point, surviving brucellae demonstrate net replication. It has been shown that increased production of ROIs by *B. abortus* infected macrophages resulted in a decreased number of recovered brucellae. These studies indicate that ROIs generated by the respiratory burst of host macrophages represent a primary defense mechanism against infection of brucellae.

The reactive oxygen intermediates are toxic to bacterial cells because they can react with proteins, lipids, and DNA and the accumulation of damaged molecules results in cell death. In bacteria, there are two mechanisms that are induced to provide resistance to oxidative killing. The primary mechanism of defense against ROIs involves enzymes such as superoxide dismutase and catalase, which work in concert to convert superoxide to hydrogen peroxide and hydrogen peroxide to oxygen and water, respectively. The secondary mechanism of defense includes enzymes involved in repair of oxidative-induced damage of proteins, lipids, and DNA. In some studies it has been suggested that at physiologically relevant concentration of ROIs and bacterial cell densities, DNA repair may play a more important role than catalase, a primary mechanism of defense against ROIs and implications are that bacterial cell death resulted from an accumulation of unrepaired DNA lesions mediated by ROIs.

In *Escherichia coli*, oxidatively damaged nucleotide bases are removed by DNA N-glycosylases, generating an apurinic/apyrimidinic (AP) site. Exonuclease III, encoded by *xthA*, can act upon these AP sites by cleaving 5' of the AP site to generate a free 3' hydroxyl group and also repair strand breaks by repairing sterically blocked 3' ends which allow DNA repair by nick translation. In *E. coli*, *xthA* mutants have been shown to be hypersensitive to exposure of hydrogen peroxide suggesting this DNA repair pathway is needed for survival in an oxidative environment.

Analysis of the recently sequenced genome of *Brucella melitensis* has revealed the presence of two *xthA* homologs in contrast to *E. coli* and *Salmonella*, which possess a single copy of *xthA*. Given that brucellae survive ROI-mediated killing by host macrophages, *xthA* is essential for repair of oxidatively damaged DNA in *E. coli*, and that brucellae survive within macrophages at a low cell density, we hypothesize that *xthA1* and *xthA2* gene products have overlapping functions and contribute to survival of *B. abortus* within host macrophages, thus contributing to its virulence *in vivo*.

POSTER # 14

P190RHOGAP-MEDIATED EFFECTS ON UPTAKE OF *YERSINIA PSEUDOTUBERCULOSIS* BY MACROPHAGES

Hudson, K.H., Bouton, A.H., Bruce-Staskal, P.J.

The *Yersinia pseudotuberculosis* outer membrane adhesin invasin binds to β1 integrin receptors present on a variety of cell types involved in the pathogenesis of Yersinia. Interaction between invasin and β1 integrins results in the clustering of integrins and activation of protein tyrosine kinases such as Src family members. Phosphorylation of signaling proteins by tyrosine kinases results in the activation of downstream effector molecules involved in actin cytoskeleton reorganization, allowing for uptake of Yersinia by both professional and non-professional phagocytes. GTPases of the Rho family are one group of downstream effector molecules that play a crucial role in regulation of the actin cytoskeleton. In this study, we have investigated the role of the upstream regulator of RhoA in mediating actin cytoskeleton reorganization upon Yersinia infection of macrophages. In order to manipulate the normal cellular levels of active GTP-RhoA, wild-type or dominant negative variants of P190RhoGap, a negative regulator of RhoA, were expressed in macrophages. Expression of both of these molecules resulted in less efficient uptake of Yersinia by macrophages as compared to mock-transfected cells. Decreased phalloidin staining was observed in macrophages expressing wild-type P190RhoGap, while increased phalloidin staining was observed in macrophages expressing dominant negative p190RhoGap. From the results of this study, we propose a model in which RhoA activity is both positively and negatively regulated during the phagocytic process to allow for effective actin cytoskeleton reorganization and thereby, efficient uptake of Yersinia by professional phagocytes.

POSTER # 15

PRODUCTION OF AN UNUSUAL EXOPOLYSACCHARIDE BY THE BOVINE PATHOGEN *HAEMOPHILUS SOMNUS.*

T. J. Inzana, [1,8], A. Cox[2], and G. Glindemann[1].
[1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA. [2]Institute for Biological Sciences, National Research Council, Ottawa, Canada.

Haemophilus somnus is responsible for multiple systemic infections of cattle, including meningoencephalitis, pneumonia, septicemia, myocarditis, and others. Most species in the genus *Haemophilus* are encapsulated, and the capsular polysaccharide (CP) is required to protect the bacterium from host defense systems in order to cause systemic disease. There has been conflicting evidence as to whether *H. somnus* produces a CP. We determined that when *H. somnus* was grown on blood agar anaerobically, but not in CO_2, a polysaccharide (PS) was produced that precipitated with the cationic detergent hexadecyltrimethylammonium bromide. This PS was also produced by *H. somnus* grown in broth cultures to late stationary phase. Preliminary electrospray-mass spectrometry and gas liquid chromatography analysis indicated that the PS was composed of mannose and galactose in a ratio of about 3:1. The PS was a heterogeneous, high molecular weight PS that was indistinguishable from CP when analyzed by SDS-PAGE. Rabbit antiserum to purified PS was produced, and ELISAs were developed to detect and quantitate the PS or antibodies to the PS. All of 23 strains of *H. somnus* tested produced an antigenically reactive PS, but the amount of PS produced varied between strains. However, the PS could not be detected by any agglutination assays, including latex agglutination. Immuno-electron microscopy indicated that the PS was not firmly attached to the cell surface, as would be expected for a CP, but was shed into the cellular matrix as an exo-PS or slime layer. Antibodies to the PS were not protective by passive immunization in a mouse model for *H. somnus* bacteremia. *H. somnus* also formed a biofilm in late stationary phase growth, but it has not yet been determined if biofilm formation is dependent on exo-PS production. In conclusion, *H. somnus* produces an exo-PS that is up-regulated under anaerobic conditions, and has unusual antigenic properties. Further investigation is warranted to determine if this PS is involved in virulence.

POSTER # 16

MUTAGENESIS OF ESCHERICHIA COLI SUGGESTS A PHYSIOLOGICAL ROLE FOR OUTER MEMBRANE VESICLES

Alexandra Johnson,* Sreekanth Vemulapalli, Caroline Hall, and Meta Kuehn
Duke University Medical Center, Department of Biochemistry, Durham NC 27710

All Gram negative bacteria studied to date produce outer membrane vesicles. Morphological and biochemical studies suggest that genes involved in vesicle production may include genes responsible for outer membrane growth and turnover. Thus vesicles may enable remodeling of the outer membrane in response to environmental stress. To understand the mechanism and function of vesicle formation, we have generated and begun to characterize mutants in vesiculation in the *Escherichia coli* strain DH5α. A transposome was used to randomly disrupt genes in the genome with a kanamycin resistance cassette. The insertion mutants were then screened for vesicle production using anti-lipopolysaccharide (LPS) immunoblotting of cell free supernatants and SDS-PAGE of crude vesicle preparations. Mutants were selected which reproducibly showed altered amounts of LPS in cell free supernatants, significantly altered amounts of outer membrane proteins (OMPs) characteristic of vesicles, and a near wild type growth rate. Genes involved in vesiculation identified in this screen include genes induced during the cold shock response. To maintain membrane fluidity during cold shock, *E. coli* incorporates unsaturated lipids into the outer membrane. To determine if vesicles contribute to membrane turnover during cold shock, vesicle production was measured from cultures at 12C and 30C. Preliminary results reveal higher vesicle production per CFU from 1 to 4 hours after cultures were shifted to 12C as compared to cultures left at 30C. These results suggest that vesicles may enable turnover of outer membrane components, allowing bacterial survival after a sudden change in environmental conditions.

POSTER # 17

THE ROLE OF THE ACCESSORY SECA2 PROTEIN IN THE LIFESTYLE OF *MYCOBACTERIUM TUBERCULOSIS*

Sherry Kurtz[1]*, Benjamin Espinosa[2], John T. Belisle[2], William R. Jacobs, Jr.[3], and Miriam Braunstein[1]

[1] Department of Microbiology and Immunology, University of North Carolina Chapel Hill, Chapel Hill, NC

[2]Department of Microbiology, Colorado State University, Fort Collins, CO

[3] Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, NY

In *Mycobacterium tuberculosis*, as in other bacterial pathogens, the proper extracytoplasmic localization of proteins is important to virulence. Mycobacteria are part of a developing group of bacteria, consisting of a number of gram-positive pathogens, which share the uncommon property of possessing two *secA* genes (*secA1* and *secA2*). The SecA protein is highly conserved throughout bacteria, and it is a central component of the general Sec-dependent protein export pathway. Using an allelic exchange strategy in *M. smegmatis*, we determined the essential nature of each *secA* in mycobacteria. The *secA1* gene is essential (like the single *secA* in *Escherichia coli*), which leads us to believe that it is the "housekeeping" *secA*. In contrast, *secA2* can be deleted and is non-essential. A phenotypic analysis of a *_secA2* mutant of *M. smegmatis* revealed a role for SecA2 in protein export. Our data indicates that SecA2 can assist SecA1 in the export of some proteins via the Sec-pathway. However, SecA2 is not the functional equivalent of SecA1 and we believe a second role for SecA2 is to export a specific subset of proteins.

In order to determine the role of SecA2-dependent protein export in pathogenesis, we deleted the *secA2* gene in *M. tuberculosis*. The virulence of the resulting *_secA2* mutant is attenuated in the mouse model of tuberculosis. In the host, *M. tuberculosis* survives within macrophages. To better understand the attenuated phenotype of the *_secA2* mutant, we are currently evaluating the ability of the *_secA2* mutant to survive and grow in bone marrow derived macrophages. By comparing the profile of proteins secreted by wild-type *M. tuberculosis* and the *_secA2* mutant, two proteins dependent on SecA2 for secretion were identified: superoxide dismutase A and catalase-peroxidase. Both of these proteins lack recognizable signal sequences at their amino-terminus and both are antioxidant proteins that may protect the bacillus from oxidative attack of macrophages. Our data suggests that SecA2 participates in a new type of secretion pathway, which is responsible for the unconventional export of proteins that promote survival of *M. tuberculosis* in macrophages.

POSTER # 18

MUTAGENESIS ANALYSES OF AGRD, THE PROPEPTIDE FOR THE AUTOINDUCING PEPTIDE (AIP) IN *STAPHYLOCOCCUS AUREUS*

Jianqun Lin*, and Guangyong Ji
Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814

The virulence gene expression in *Staphylococcus aureus* is under the control of the accessory gene regulator (*agr*). This locus consists of two divergent operons transcribed from the P2 and P3 promoters. The P2 operon encodes four genes: *agrA*, *B*, *C* and *D* whose products assemble a quorum sensing system with AgrA and AgrC as a two-component signal transduction system and AgrD as a propeptide that is processed by AgrB to generate the autoinducing peptide (AIP) which interacts with AgrC and subsequently activates the signal transduction system. The function of this quorum sensing system is to positively regulate the transcription of both the P2 and P3 operons. The P3 transcript, RNAIII, is the actual regulator that controls the virulence gene expression. Four groups of *S. aureus* have been identified based on the *agr* sequences and the group specific interactions between AIP and AgrC, and between AgrB and AgrD. The processing of AgrD propeptide involves several events: the proteolytic digestion at two sites within AgrD, the formation of an intramolecular thioester bond and the secretion of mature AIP. In this study, a set of group I AgrD mutants were made and used to determine the minimum sequence requirement as a substrate for group I AgrB and to identify the critical amino acid residues involved in the processing events. Deletion mutation analyses of AgrD from either the N- or the C-terminus revealed that the minimum sequence required for AgrD to be processed by AgrB to generate mature AIP were from amino acid residue 11 to 42. Two putative group I AgrD processing motifs, Ala23-Ala24 (AA) and Asp33-Glu34 (DE, these two residues are highly conserved among the AgrD homologues), were changed by site-directed mutagenesis to AG, GA or GG, and DD, EE or ED, respectively. Our results suggested that group I AgrB hydrolyzed ester bond at the carboxylic side of either alanine or glycine but residue Ala23 also played an important role in the recognition by group I AgrB. Similarly, group I AgrB hydrolyzed ester bond at the N-terminal side of aspartic acid residue but weakly at the N-terminal side of glutamic acid residue. However, the group I AgrD G23G24 mutant could not be processed by group II AgrB even though group II AgrB hydrolyzes ester bond at the carboxylic side of G23 of group II AgrD, suggesting that the other residues around the AgrD N-terminal processing site were important for the recognition of AgrD by AgrB. Mutation of Gly20 to Ala20, the only conserved residue preceding the N-terminal AgrD processing site among all the AgrD homologues identified, had no effect on the mature AIP production. Mutations of Pro28 or Glu40 to Ala38 or Ala40, the conserved residues following the C-terminal AgrD processing site, greatly reduced AIP production, that suggested that these residues were also important for the recognition of AgrD by AgrB.

POSTER # 19

GENERATION AND CHARACTERIZATION OF OUTER MEMBRANE VESICULATION MUTANTS IN *ESCHERICHIA COLI*

Amanda J. McBroom*, Alexandra P. Johnson, Meta J. Kuehn
Duke University Medical Center, Department of Biochemistry, Durham, NC 27710

Outer membrane vesicles are generated by all Gram-negative bacteria studied to date, including *Escherichia coli.* Outer membrane vesicles are involved in a variety of bacterial processes including growth, communication, and virulence factor transmission, however little is known about the process by which outer membrane vesicles are formed. To study the mechanisms involved in vesicle production and further elucidate the functions of vesicles, we conducted a mutagenesis screen to identify mutants that either over- or under-produced vesicles. Mutagenesis of a laboratory DH5α *E. coli* strain was carried out by random genomic insertion of a transposon carrying a kanamycin resistance cassette. Vesiculation mutants were identified from the Kanamycin transposon mutants by screening small-scale vesicle preparations with an outer membrane antibody. Larger vesicle preparations of candidate mutants from the primary screen were further characterized for vesiculation phenotype using densitometric analysis of selected outer membrane proteins. The disrupted genes were identified in those mutants found to reproducibly release an increased or decreased quantity of vesicles in comparison to wild type DH5α. The vesiculation mutants ranged from 0.4 to 0.7-fold less than wild type and 3 to 190-fold more than wild type. Mutants were further characterized by detergent sensitivity, membrane leakiness, and growth phenotypes. There was not a distinct correlation between detergent sensitivity, leakiness, viability, and vesiculation, demonstrating that vesicle production is not linked to cell lysis. Further characterization of these mutants will contribute to understanding how vesicle production is linked to microbial pathogenesis.

POSTER # 20

ANALYSIS OF THE TWIN-ARGININE (TAT) PATHWAY IN *MYCOBACTERIUM TUBERCULOSIS*

Justin A. McDonough[1], Anthony Flores[2], Martin S. Pavelka, Jr.[2], and Miriam Braunstein[1]
[1]Department of Microbiology & Immunology, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7290
[2]Department of Microbiology & Immunology, University of Rochester, Rochester, NY

Tuberculosis claims more lives per year than any other infectious disease. The causative agent, *Mycobacterium tuberculosis*, relies heavily on protein secretion pathways to properly localize important virulence factors. In addition to the well-studied Sec-dependent export pathway, another system exists in bacteria to transport proteins across the cytoplasmic membrane. This pathway, the twin-arginine translocation (Tat) pathway, transports proteins bearing an amino-terminal signal sequence containing a twin-arginine motif. Notably, substrates of the Tat pathway are exported across the cytoplasmic membrane in a folded state. Studies in the pathogenic bacterium, *Pseudomonas aeruginosa*, demonstrate the importance of the Tat pathway in exporting virulence factors such as phospholipase C and oxidoreductases involved in anaerobic growth[3]. We analyzed the genome of *M. tuberculosis* and found homologues to components of the twin-arginine translocase of other bacteria, and putative Tat-dependent substrates including four phospholipase C enzymes. An unexpected finding was that BlaC, a β-lactamase enzyme, normally secreted by the Sec-dependent pathway in other bacteria, has a putative Tat signal sequence in *Mycobacteria.* β-lactamase is only active upon export, where it is involved in hydrolyzing antibiotics such as ampicillin. BlaC of *M. tuberculosis* confers resistance to β-lactam antibiotics.

We hypothesize that the Tat pathway in *M. tuberculosis* is involved in exporting enzymes involved in anaerobic growth, virulence factors such as phospholipase C, and β-lactamase. Our characterization of the Tat pathway in *M. tuberculosis* involves two independent approaches. First, to identify Tat-secreted proteins, we have created a series of plasmids expressing either known classical Sec signal sequences or putative Tat signal sequences from *M. tuberculosis* proteins fused to the truncated BlaC. The truncated BlaC protein lacks its endogenous signal sequence. Expression of these plasmids in a *Mycobacterium smegmatis* β-lactamase mutant strain allows us to utilize BlaC as a reporter for export by screening for ampicillin-resistant bacteria. Our preliminary data suggest that the Tat pathway alone exports BlaC, independent of the Sec pathway. Thus the BlaC reporter can help identify proteins with functional Tat signal sequences. Our second approach involves constructing a *M. tuberculosis tatC* mutant, which we will use to test for attenuation in virulence in both a macrophage and mouse model of infection. These approaches will allow us to understand the role that the Tat pathway plays in *M. tuberculosis* pathogenesis, and identify proteins secreted by the Tat pathway in *M. tuberculosis.*

[3]Ochsner, U.A., Snyder, A., Vasil, A.I., and Vasil, M.L. (2002) Effects of the twin-arginine translocase on secretion of virulence factors, stress response, and pathogenesis. *Proc Natl Acad Sci USA* 99:8312-7.

POSTER # 21

ENTEROPATHOGENIC *ESCHERICHIA COLI* ESPF: BIOCHEMICAL CHARACTERIZATION AND INTRACELLULAR LOCALIZATION.

Jean-Philippe Nougayrède and Michael S. Donnenberg
University of Maryland, School of Medicine, Division of Infectious Diseases, Baltimore MD.

The mechanisms by which enteropathogenic *Escherichia coli* (EPEC), an important cause of diarrhea among infants in developing countries, induce symptoms are not yet completely understood. EspF is one of the EPEC effector proteins translocated by the type III secretion system into host cells. It bears proline-rich repeats which resemble those recognized by eukaryotic proteins containing SH3 domains. EspF is required for the full impact of EPEC on disruption of host barrier function. EspF has also been shown to trigger apoptosis in transfected cells, and an EPEC *espF* mutant is deficient in host cell killing. In an effort to understand the function of EspF, we are pursuing its biochemical characterization and the identification of its target(s) in host cells.

To enhance yield and purity, a C-terminal hexahistidine tagged EspF was overexpressed in BL21[DE3] under T7 promoter control in an artificial operon with its chaperone CesF. Native purification by Ni-agarose affinity chromatography yielded the chaperone and two forms of EspF: a 28-kDa form lacking the first methionine, and a 21-kDa form blocked at its N-terminus which stained poorly with Coomassie blue. The molecular mass of the two EspF forms was determined by MALDI-TOF, and was found to be higher (~ 550 Da) than the theorical masses of the corresponding EspF fragments. EspF thus appears to be post-translationally modified. In addition, secondary structure prediction indicated that EspF is likely to be largely unfolded. The EspF/CesF complex was poorly soluble and precipitated/autoaggregated spontaneously at low temperature.

In silico analysis of EspF predicted a >0.95 probability of export to mitochondria. EspF was demonstrated by immunofluorescence in infected cultured cells using monoclonal antibodies. EspF showed a punctate pattern, and was shown to co-localize with a mitochondrial fluorescent probe. In addition, a truncated (possibly processed) form of EspF could be detected by western blot in the mitochondrial fraction of infected cells, further suggesting that EspF is addressed to mitochondria. We are currently investigating the influence of EspF on mitochondrial function and apoptosis induction.

POSTER # 22

PERFRINGOLYSIN O AND ALPHA TOXIN ARE REQUIRED FOR SURVIVAL OF *C. PERFRINGENS* IN HOST TISSUE

David K. O'Brien and Stephen B. Melville
Department of Biology, Virginia Tech, Blacksburg, Virginia.

Clostridium perfringens is a Gram-positive anaerobic pathogen, which causes a variety of diseases in humans, including gas gangrene (clostridial myonecrosis). Of the 13 different toxins produced by *C. perfringens*, two are thought to be important in the pathogenesis of gas gangrene, alpha toxin (PLC) and perfringolysin O (PFO), encoded by the *plc* and *pfoA* gene respectively. In a previous report, it was discovered that *C. perfringens* could escape the phagosome and survive in the presence of the mouse macrophage-like cell line J774-33, even under aerobic conditions. However, the mechanism explaining how *C. perfringens* could survive in the presence of macrophages was unknown. To determine if PLC and PFO play a role in survival inside macrophages, homologous recombination was used to construct a *pfoA-* mutant of *C. perfringens* strain 13. A *plc-* mutant of strain 13 was obtained and used as the host strain to construct a *plc-/pfoA-* mutant strain. Using these mutant strains, we found that *C. perfringens* survival in the presence of J774-33 cells is dependent on PFO but not PLC. In contrast, the ability of *C. perfringens* to survive in the presence of mouse peritoneal macrophages is dependent on both PFO and PLC. The ability of *C. perfringens* to escape the phagosome of J774-33 cells and mouse peritoneal macrophages is mediated by either PFO or PLC; when both toxins are absent *C. perfringens* remains inside phagosomes. Using a mouse model we also found that PFO or PLC was necessary for *C. perfringens* to survive in vivo at doses 1000 times lower than those required to initiate a gangrene infection. We found that while J774-33 cells are more susceptible to *C. perfringens* dependent cytotoxicity than mouse peritoneal macrophages, both cell lines are more sensitive to PFO expressing strains. This is the first report of a role for PFO in in vivo conditions. We propose that PFO and PLC play a critical role in the survival of *C. perfringens* during the important early stages of gangrene infections, when phagocytic cells are present and bacterial numbers are low.

POSTER # 23

SEPL, A NON-STRUCTURAL COMPONENT OF THE ENTEROPATHOGENIC *E. COLI* TRANSLOCATION APPARATUS.

Colin B. O'Connell

Enteropathogenic *Escherichia coli* (EPEC), a cause of infantile diarrhea in the developing world, disrupts host cell microvilli, causes actin rearrangements, and initiates the formation of a cup-like pedestal to which the bacteria intimately adhere. This process, termed the attaching and effacing effect (A/E), is considered to be the hallmark of EPEC infection. The genes required for A/E have been localized to a 36 Kb pathogenicity island called the locus of enterocyte effacement (LEE), which is sufficient to allow non-pathogenic *E. coli* to form A/E lesions. The *sepL* gene of the LEE is predicted to encode a protein with a mass of approximately 39 kDa. Using the *sepL* mutant strain UMD878, we report that a functional *sepL* gene is required for A/E. To determine the effect of SepL on secretion, a series of western analyses was performed. These studies show that the mutant strain secretes greatly reduced amounts of the translocation apparatus proteins EspB, EspD and EspA, while the secretion of effector proteins Tir, EspF and EspG remains at wild type levels. This variation from wild type levels was quantified by a serial dilution assay, which showed that EspB is secreted 162-fold less in UMD878 as compared to wild type, while EspF is secreted at wild type levels. An EspF-*cya* fusion protein was used to demonstrate that the mutant strain is unable to translocate EspF into the host cell. This translocation defect was also apparent when the translocation of phosphorylated Tir was examined via western blot. Cell fractionation studies indicated that SepL is not secreted and that it is a cytoplasmic. The differential effect of SepL on secretion of translocation apparatus and effector proteins, the role of SepL in translocation, and the location of SepL in the cytoplasm indicate that SepL is a non-structural component of the EPEC translocation apparatus and thus has a novel role in type III secretion. Recent data generated by other laboratories has indicated a potential interaction between SepL and SepD (*rOrf6*). Current studies are attempting to confirm these finding and hope to shed some light on the role of this interaction in SepL function.

POSTER # 24

ANALYSIS OF THE REGULATION OF THE VIRULENCE REPRESSED GENES OF *BORDETELLA PERTUSSIS* BY BVGR AND RISA

Tadhg Ó Cróinín, Vanessa Grippe and Tod Merkel
Laboratory of Respiratory and Special Pathogens, CBER, FDA
Bethesda, MD.

Bordetella pertussis, the causative agent of whooping cough uses a wide array of virulence factors to successfully colonize the respiratory tract and cause disease. The expression of many of these factors is activated by the BvgAS two-component regulatory system. In addition, this two-component regulatory system is responsible for the repression of a second family of genes, designated the *bvg*-repressed genes through induction of the repressor BvgR. More recent studies have revealed a second two component regulatory system designated RisAS which appears to play a role in induction of the *bvg*-repressed genes. The aim of this study was to characterize the regulation of the *bvg*-repressed genes by the BvgR and the RisAS regulatory systems. Isogenic mutants, bearing in-frame deletions of the b*vg*R and *ris*A genes, were created. Various sets of *lacZ* fusions to different sized deletion fragments of the promoter regions of *bvg*-repressed genes were constructed and inserted into the wild-type and mutant backgrounds. These fusions were used to identify putative binding sites of both BvgR and RisA and for the analysis of the regulation of the *bvg*-repressed genes by BvgR and RisA under a variety of different environmental conditions. Using these fusions we have demonstrated repression of the *bvg*-repressed genes by BvgR and identified a putative binding site for this protein. Furthermore we have demonstrated a role for RisA in the induction of the *bvg*-repressed genes suggesting that these genes are regulated by both the Bvg and Ris two-component regulatory systems.

POSTER # 25

REGULATION OF TRANSCRIPTION OF THE INVASION GENE ACTIVATOR, *HILA* OF *SALMONELLA ENTERICA* SEROVAR *TYPHIMURIUM*

Igor N. Olekhnovich and Robert J. Kadner
Department of Microbiology, School of Medicine, University of Virginia,
Charlottesville,Virginia 22908-0734

Salmonella enterica serovar Typhimurium is a facultative intracellular pathogen that causes gastroenteritis in humans and systemic disease similar to typhoid in mice. Its ability to invade host cells depends on genes encoding a type III secretion system found in *Salmonella* pathogenicity island 1 (SPI1) as well as effector protein injected by that secretory machinery. Expression of most of these genes is believed to be controlled by numerous environmental signals, including osmolarity, oxygen level and grows phase, all of which combine to influence expression of the major SPI1-encoded transcriptional regulator HilA. Many genes that affect *hilA::lacZY* reporters *in vivo* include positive elements *hilC/sirC/sprA, hilD, sirA, pstS, fis, barA, csrAB, phoB, fadD*, and *fliZ* as well as negative elements such as *phoPQ, hha, lon, hilE, pag, and ams*. It has been suggested that HilD, HilC and Hha directly regulate *hilA* expression, because these proteins have been shown to bind to the *hilA* promoter.

To begin to dissect the complex cascade of direct and indirect regulatory factors controlling HilA levels, we expressed in *E. coli* and purified three *S.* typhimurium proteins: PhoP, SirA, and Hha, for comparison with the HilC and HilD proteins. Each protein was tested in a range of concentrations in DNA-binding mobility shift assay with different fragments of *hilA* promoter and *in vitro* transcription experiments. Only Hha, HilC and HilD were able to shift DNA fragment containing the *hilA* promoter. The Hha protein inhibited *hilA* transcription *in vitro*. The effect of the HilD/C proteins on Hha-*hilA* promoter interaction and transcription control is shown.

In another approach to identify additional regulatory proteins that might affect hilA expression, we looked for proteins that interact with the HilD/C proteins and might be involved in their modification. Thus, certain proteins from *E. coli* cells were found to routinely copurify with immobilized His-tagged versions of HilC and D, when they were expressed in this host. Four proteins that were selected for analysis were those that were copurified with both His6-HilD and His6-HilC. By means of in-gel tryptic digestion and mass spectrometric analysis performed by the Biomolecular Research Facility at University of Virginia, the proteins were identified as DnaK, GroEL, GluS, and UDP-glucuronate dehydrogenase. It is doubtful that the association of these proteins with the HilC and HilD proteins represents a specific regulatory input.

POSTER # 26

HOST CELL-PATHOGEN INTERACTIONS: A ROLE FOR HOST TYROSINE KINASES FAK AND PYK2 DURING *YERSINIA PSEUDOTUBERCULOSIS* INFECTION

Kate Owen, Pamela J. Bruce-Staskal and Amy H. Bouton

Efficient entry into host cells by the enteropathogen *Yersinia pseudotuberculosis* is mediated through high affinity interactions between the bacterially encoded invasin protein and β1 integrins on host cells. Upon receptor ligation, focal adhesion kinase (FAK) is recruited to the site of adhesion where it becomes activated. Proline rich tyrosine kinase 2 (Pyk2), a second focal adhesion kinase family member expressed in haemopoetic cells, also becomes activated during integrin stimulation. However, despite homology to FAK, the role of Pyk2 in signaling pathways is thought to be distinct from that of FAK. In order to better understand the relationship between these two signaling molecules, and gain insight into the role they play during bacterial uptake, we first investigated whether FAK and Pyk2 become activated following invasin-β1 integrin interactions. Upon Yersinia infection in adherent macrophages, Pyk2 became highly activated, while FAK phosphorylation levels remained constant. However, the activation of Pyk2, in response to Yersinia infection, appeared to be independent of invasin expression. Second, we investigated whether prior engagement of β1 integrins with extracellular matrix molecules affected the ability of FAK and Pyk2 to become activated during infection. Interestingly, Yersinia infection resulted in the generation of a distinct set of signals from those initiated through adhesion events. Both FAK and Pyk2 appeared to be dephosphorylated in Yersinia-infected macrophages held in suspension, a result that differed from adherent cells similarly infected. We therefore propose that FAK and Pyk2 are differentially regulated, but that both signaling molecules can act in a coordinated fashion depending on the stimulus received through β1 integrin interactions.

POSTER # 27

IDENTIFICATION OF VIRULENCE DETERMINANTS IN *STREPTOCOCCUS SANGUIS* BY SIGNATURE-TAGGED MUTAGENESIS.

Sehmi Paik[1], Jody C. Noe[1], Cindy L. Munro[2], and Todd Kitten[1];
The Department of Microbiology and Immunology and The Philips Institute[1], and the Department of Adult Health Nursing[2], Virginia Commonwealth University, Richmond, VA.

Streptococcus sanguis belongs to the viridans group of oral streptococci, which cause 40-50% of native valve endocarditis in humans. Several virulence factors for infective endocarditis have been evaluated previously. However, the identification of new virulence factors is important to facilitate new drug treatment and vaccine development. It is believed that infective endocarditis occurs when previously damaged heart valves are exposed to transient bacteremia. Oral streptococci can enter the bloodstream through invasive dental procedures and even tooth-brushing and chewing. *S. sanguis* is the most common cause of endocarditis among the viridans streptococci. Therefore, *S. sanguis* was chosen for identifying virulence determinants for endocarditis using a modified transposon mutagenesis technique, called signature-tagged mutagenesis (STM). A *mariner*-based mini-transposon was modified by insertion of signature tags comprised of a central variable DNA sequence and invariant flanking ends. Forty unique tagged transposons were selected and used to create signature- tagged mutants by *in vitro* transposition and subsequent transformation of *S. sanguis*. To identify mutant strains with increased or decreased virulence, a rabbit endocarditis model was used. Rabbits were catheterized using a rigid material to traumatize the aortic valves. Two days later, rabbits were inoculated with 10^8 cells derived from a pool of 40 mutant strains with unique signature tags (the input pool) and were subjected to necropsy the next day. The vegetations were excised, homogenized in PBS, and plated on agar plates to recover the bacteria that successfully survived at the site of infection (the output pool). DNA was extracted from the input and output pools and the signature tags amplified by PCR. The PCR products were labeled and hybridized to a membrane containing the original signature tags in a dot blot format. The results were compared to identify candidate avirulent or hypervilrulent mutants. Currently, we have tested about 720 signature-tagged mutant strains and identified 28 putative avirulent mutants as well as 3 putative hypervirulent mutants. Characterization of genes disrupted by transposon insertion in these mutants is underway.

POSTER # 28

IS THE *FDT* OPERON OF *BRUCELLA ABORTUS* 2308 INVOLVED IN THE TRANSPORT OF FERRIC-2,3-DIHYDROXYBENZOIC ACID UNDER CONDITIONS OF HIGH IRON REQUIREMENT.

James T. Paulley$_1$, Bryan H. Bellaire$_2$, and R. Martin Roop II$_1$,
1Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, NC 27858-4354, 2Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, Louisiana 71130.

Iron is essential for life in all organisms. Therefore, the ability for bacteria to acquire iron in iron-limiting conditions is essential for their growth and development. One of the ways that bacteria cope with iron deprivation is through the production and excretion of siderophores. These low molecular weight, high affinity iron binding compounds bind extracelluar iron and deliver it back to cell through specific transport systems. *Brucella abortus*, a gram negative intracellular pathogen, produces the monocatechol siderophore 2,3-dihydroxybenzoic acid (DHBA) in response to iron limitation. Studies employing a genetically defined *B. abortus* mutant unable to produce DHBA have shown that the production of DHBA is required for virulence in pregnant cattle, the natural ruminant host. *In vitro* studies suggest that the attenuation of the *B. abortus* DHBA-deficient mutant is based on its inability to acquire sufficient iron to fuel the efficient metabolism of erythritol. This four carbon sugar alcohol is the preferred carbon source of *B. abortus* and is found at high levels in the reproductive tract of pregnant ruminants during the latter stages of gestation.

A survey of the recently released genome sequence of *B. melitensis* 16M revealed the presence of a cluster of putative genes that appear to encode a DHBA transport system. These genes have been designated *fdt* (ferric DHBA transporter) A, *fdt*B, *fdt*D, and *fdt*C, and are predicted to encode a TonB-dependent outer membrane receptor, a periplasmic binding protein, one cytoplasmic membranes permease, and a cytoplasmic membrane associated ATPase, respectively. To confirm the participation of FdtA in DHBA transport, an isogenic *fdt*A mutant (JTP1201) was constructed from virulent *B. abortus* 2308 by gene replacement. This mutant is presently being evaluated *in vitro* for its capacity to utilize DHBA as a siderophore.

POSTER # 29

TRANSPORT AND ACTIVITY OF PERTUSSIS TOXIN IN MAMMALIAN CELLS

Zoë E.V. Phillips* and Nicholas H. Carbonetti
Department of Microbiology and Immunology,University of Maryland School of Medicine, Baltimore, MD 21201, USA.

A number of respiratory tract infections occur as the result of infection with gram-negative bacteria from the *Bordetellae*. In humans, *Bordetella pertussis* is the causative agent of a disease called Whooping cough or Pertussis, where a range of symptoms, including a paroxysmal cough, are observed. Pertussis toxin is an important virulence factor for this organism (PT^- mutants have a reduced ability to colonize the host organism or cause damage therein). PT is a multimeric protein of six subunits, two being identical, organized in an AB_5 structure. The holotoxin encompasses a pentameric ring of B subunits associated with a pyramid shaped A subunit (also called S1). The S1 subunit bears the enzymatic activity. PT is an ADP-ribosyltransferase that modifies several mammalian heterotrimeric G proteins, resulting in a wide range of effects within the cell. Little is known regarding PT transport to its target proteins within mammalian cells. However a number of bacterial toxins are believed to undergo retrograde intracellular transport through the Golgi apparatus to the endoplasmic reticulum. Constructs containing the S1 subunit of PT, with and without a signal peptide that targets to the ER, were used to transfect CHO cells to gain further evidence that PT traffics through the ER as part of the cellular intoxication pathway. Both bacterial and eukaryotic signal peptides were tested. ADP-ribosylation of target G proteins is brought about by S1 when expressed in stable transfectants, even when directed to the ER by a signal peptide. When coupled to a signal peptide PT localizes to the perinuclear region whereas, S1 with no signal peptide was found diffusely throughout the cell. However in stable transfectants, S1 expression levels are significantly greater than cell-associated levels observed when treated with exogenous PT. We are currently engineering constructs placing S1 under control of a tetracycline-inducible promoter, this should allow regulation of S1 expression, to obtain a level approaching that observed in cells treated with PT exogenously. The ERAD pathway involves misfolded protein removal and degradation, it is considered a possible pathway exploited by the S1 subunit of PT for transport from the ER to the cytosol. This pathway utilizes the Sec61-associated translocation channel for return of proteins to the cytosol. Once in the cytosol these proteins are ubiquitinated at lysine residues, the major signal for targeting proteins to the proteasome for degradation. For PT to utilize this pathway the protein must presumably avoid ubiquitination and degradation. S1 contains no lysine residues perhaps allowing avoidance of ubiquitination and its consequences. We engineered a number of mutant strains of *B. pertussis* replacing one, two or three arginine residues with lysine residues, in a variety of locations, to examine whether addition of lysine reduces PT toxicity by allowing ubiquitination and degradation to occur. More than one lysine was substituted in a number of mutants since it is possible that the signal or trigger for ubiquitination may require more than one lysine residue. Several mutants have been identified that have *in vitro* enzymatic activity equivalent to wildtype but reduced cellular activity.

POSTER # 30

WHY ARE THE PHOSPHOTIDYLCHOLINE PHOSPHOLIPASE C AND SPHINGOMYELINASE GENES SILENT IN *BACILLUS ANTHRACIS*?

Andrei P. Pomerantsev[1], Manuel Osorio[2], Kirill V. Kalnin[1] and Stephen H. Leppla[1]
[1]National Institute of Allergy and Infectious Diseases, NIH, and [2]Center for Biologics Evaluation and Research, FDA, Bethesda, MD

The genomes of *Bacillus anthracis* and *Bacillus cereus* contain highly similar *plc* and *sph* genes encoding the active phosphotidylcholine phospholipase C (PC-PLC) and sphingomyelinase (SPH), respectively. However, in *B. anthracis* these genes are silent. Expression of both genes in *B. cereus* is under the control of the global transcriptional regulator PlcR. We have shown that PlcR is a positive regulator of *plc* but negatively regulates *sph*. On the other hand, PlcR activity can be regulated by PapR, the putative product of a short open reading frame located downstream of *plcR*, and possibly also by neutral protease B, the product of *nprB*, which is located immediately upstream of *plcR*. The fact that both PapR and NprB contain signal peptides whereas PlcR does not, presents a dilemma of how two extracellular proteins may regulate the activity of one that resides intracellularly. We propose that an oligopeptide permease may help recruit the secreted proteins or their products back into the cell. The genome of *B. anthracis* contains a truncated and inactive version of *plcR*, an intact *papR* gene and a complete cluster of oligopeptide permease genes. However, the *B. anthracis* genome does not contain the *nprB* gene. Replacement of the *B. cereus plcR* gene by the inactive *B. anthracis* orthologue eliminated both PC-PLC and SPH activities. However, the introduction of the *B. cereus nprB- plcR- papR* gene cluster into *B. anthracis* as well as overexpression of *B. cereus plcR* did not activate *B. anthracis* PC-PLC or SPH. We found that a translational fusion of PlcR with PapR gave strong activation of these enzymes in *B. anthracis*. Interestingly, expression of *B. cereus nprB* in *B. anthracis* was lethal to the host cells. Because *B. cereus* is considered a predecessor of *B. anthracis*, we suggest that the introduction of the virulence plasmids, pXO1 and pXO2, into *B. cereus* resulted in a compensating elimination of the *nprB* gene and a point mutation in *plcR*. These few changes may account for the many differences in the phenotypic and pathogenic properties of these two otherwise highly similar bacteria.

POSTER # 31

THE MYST-FAMILY HISTONE ACETYLTRANSFERASE OF *ENTAMOEBA HISTOLYTICA*

Girija Ramakrishnan, Barbara J. Mann, and William A.Petri,Jr.
Depts. of Medicine, Microbiology and Pathology, Univ. of Virginia, Charlottesville, VA 22908, USA

Our laboratory has been studying mechanisms of transcriptional regulation as a determinant of pathogenicity in *Entamoeba histolytica.* In light of the small genome size, it would also be interesting to look at the repertoire of transcription factors in *E. histolytica* relative to that of a typical metazoan, or even of another protozoan parasite.

One primary conserved mechanism of transcriptional activation in eukaryotes is the acetylation of histones in the chromatin. Histone acetyltransferases (HATs) are ubiquitous proteins and are grouped in distinct families. Members within a family show high sequence conservation. Using a *Drosophila melanogaster* sequence belonging to the MYST family of HATs in BLAST analysis, we identified the corresponding homolog (ehMYST) in the *E. histolytica* genome. In a BLAST search against the Swissprot database, the ehMYST ORF gave high scores with various members of the MYST family of HATs, including human MOZ, *Drosophila* MOF, and yeast (*Saccharomyces cerevisiae*) Esa1. The characteristic features of the family are conserved in ehMYST. The MYST family HATs form multiprotein complexes in vivo and have specificity for histones H4 or H3. They function as co-activators of gene expression in a variety of contexts. In order to characterize the ehMYST protein, we have expressed it as a $(His)_6$ and FLAG-tagged protein in *E. coli.* In preliminary experiments, the protein appears to have histone acetyltransferase activity for a peptide corresponding to the histone H4 amino-terminus. We are in the process of further characterizing the enzymatic activity of this protein and its role in *E. histolytica.*

POSTER # 32

ROLE OF THE SOS REGULON IN THE PATHOGENESIS OF *BRUCELLA ABORTUS*.

Christelle Roux. R. M. Roop* and Don G. Ennis
Department of Biology, University of Louisiana, Lafayette, LA 70504. *Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, NC 27858.

Brucella abortus is a Gram-negative facultative intracellular pathogen responsible for abortions and infertility in animals and undulant fever in humans. Recent research has indicated that the SOS DNA repair and recombination functions may play important roles in virulence of certain intracellular pathogens, specifically in surviving the oxidative damage within professional phagocytes. Very little is known about DNA repair and recombination networks of *B. abortus*. To assess their possible role(s) in pathogenesis, we are cloning and characterizing a number of these genes. Previously a *recA* gene from *B. abortus* was identified from which a *recA*-disruption mutant was constructed; however, this *recA* mutant exhibited only modest sensitivity to UV irradiation. We are investigating the existence of a second RecA-like protein to explain this observation. Using Western Immunoblots, we detected a RecA-like protein at the expected molecular weight from cell extracts of the *recA*-deleted strain. This "second" RecA protein was induced following exposure to DNA damage. Low stringency Southern hybridization assays also suggested a "second" divergent *recA* gene. These results are consistent with the notion that *B. abortus* carries multiple *recA* genes. Our efforts to clone this putative second *recA* gene using functional selection from phage and plasmid libraries or degenerate primers remained unsuccessful. A blast search in the genome of *B. abortus* suggested homologies between the RecA and a distantly related protein called RadA, an archeal homologue also involved in homologous recombination. We are now cloning the *radA* gene and we will investigate the role of this archeal protein in the survival of *B. abortus* to DNA damaging agents.

We are also investigating the effect of a non-cleavable *E. coli* LexA mutant protein in *B. abortus*. Surprisingly, wild type and repair-deficient mutants of *B. abortus* showed an increased sensitivity to DNA damaging agents such as mitomicyn C and methylmethane sulfonate. The survival of these strains in murine resting or IFN-γ activated macrophages is in progress.

POSTER # 33

THE BACTERIAL ADAPTIVE RESPONSE GENE, *BARA*, IS A GLOBAL VIRULENCE FACTOR REGULATING MOTILITY, ATTACHMENT AND SURVIVAL IN *ESCHERICHIA COLI.*

Surashri Nandan Sahu, Sharmistha Acharya, Isha Patel, Helina Tuminarom and Suman Mukhopadhyay*.
Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland, College Park, MD-20742.

Bacterial adaptation to environmental changes depends to a greater extent on two-component signal transduction systems. They consist of a membrane bound sensor kinase, which is responsible for detection of environmental changes and its cognate response regulator that is phosporylated by the kinase for subsequent signal transduction and modulation of cellular functions. The *Escherichia coli* genome sequence indicates that there are approximately 30 typical two-component systems. One such sensor is the bacterial adaptive response kinase, BarA, encoded by the *barA* gene. BarA was initially identified as a multicopy suppressor of *envZ* mutation modulating the outer membrane protein expression. BarA also have been implicated in the oxidative stress response survival and regulation of transcription of the alternate sigma factor *rpoS*. The survival of a *barA* mutant strain, but not a *rpoS* mutant, within human U937 macrophages was severely compromised indicating that BarA may regulate stress response pathway independent of RpoS. Similarly, a *barA* mutant, but not a *rpoS* mutant, exhibited much reduced motility in swarming assays. The attachment to epithelial cells was severely reduced in absence of functional BarA, however complementation with a functional clone only partially restored cellular attachment and survival. The rate and quantity of biofilm formation was four fold lower on glass and polyvinylchloride surface in the absence of *barA* gene but not in an isogenic *rpoS* mutant known to have moderate biofilm formation defect. A comparison of scanning electron micrographs indicates that *barA* may regulate synthesis/assembly of cellular appendages and exopolysacharide production (also as confirmed by ruthenium red staining). The synthesis of several outermembrane proteins appears to be regulated by BarA signaling pathway confirming the ultrastructure studies, however their identity remains unknown. Gene profiling asssays using cDNA microarrays reveal that BarA regulates biosynthesis of pili, flagella, polysacharide and cell division in *E. coli*. Taken together, these results suggest that BarA signaling regulates survival, motility and attachment demonstrating that BarA sensor kinase regulates virulence in *E. coli*.

POSTER # 34

SENSITIVE, REAL-TIME PCR DETECTS LOW-LEVELS OF CONTAMINATION BY *LEGIONELLA PNEUMOPHILA* IN COMMERCIAL REAGENTS

Hua Shen[1], Snezna Rogelj[2], Thomas L. Kieft[2]
[1] Biology Department of Virginia State University, Petersburg, VA
[2] Biology Department, New Mexico Tech, Socorro, NM

In a quantitative investigation of *Legionella pneumophila* from swamp cooler water in Socorro, New Mexico, we could detect as little as one bacterium in a sample using a real-time PCR method targeting the *L. pneumophila*–specific *mip* gene. In addition to finding relatively high numbers of *L. pneumophila* in swamp cooler waters, we also detected low levels of fluorescent PCR signal and DNA product examined by gel electrophoresis in our negative controls. A further control experiment using deionized, filter-sterilized water ruled out the likelihood of contamination being introduced during experimental procedures. The contamination persisted when three separate batches of PCR reagents and water from two different biochemical companies were tested, suggesting that the contamination was in the PCR reagents used. Among these, water is the number one suspect because it accounts for 80% of the volume of the reaction. To further verify that the contamination was indeed *L. pneumophila*, we subcloned the DNA fragment from the negative control, and the DNA sequence was determined to be *L. pneumophila mip* DNA. Since *L. pneumophila* is widespread in aqueous environments, forms biofilms in water distribution pipelines and storage vessels, and is resistant to biocide, it is possible that commercially available purified water and PCR reagents harbor low level contamination of *L. pneumophila* DNA that have escaped purification processes. This presents a challenge when developing sensitive DNA-based bacterial detection method if the target organism or its DNA are common contaminants of necessary reagents.

POSTER # 35

PATTERNS OF OPACITY PROTEIN EXPRESSION AMONG VAGINAL ISOLATES OF *NEISSERIA GONORRHOEAE* OVER THE COURSE OF EXPERIMENTAL MURINE GENITAL TRACT INFECTION

Amy N. Simms and Ann E. Jerse
Department of Microbiology and Immunology, Uniformed Services University
Bethesda, Maryland 20814-4799

The opacity (Opa) proteins of *Neisseria gonorrhoeae* are a family of antigenically variable outer membrane proteins that are hypothesized to mediate intimate attachment of gonococci (GC) to epithelial cells and non-opsonic uptake by neutrophils. The expression of individual *opa* genes is phase variable due to a frame shift mechanism that occurs in a repeated region within each gene. The kinetics of Opa protein expression within the urogenital tract have been described in a human urethritis model and in a mouse model of lower genital tract infection. In these studies, male volunteers or female mice were inoculated with a predominantly Opa-negative population of GC. Within 48 hours post inoculation, a predominance of Opa-positive GC was recovered. It is not known whether the recovery of Opa-positive GC was due to selection of a pre-existing population of Opa-positive GC or to induction of *opa* gene phase variation. Here we further explored the kinetics of Opa protein expression in vivo by determining the Opa phenotype of vaginal isolates from mice inoculated with defined mixtures of Opa variants of strain FA1090. In two experiments, mice were inoculated with suspensions containing primarily Opa-negative and Opa1-expressing GC (Experiment 1: 75% Opa-negative, 25% Opa1; Experiment 2: 55% Opa-negative, 45% Opa1). The Opa phenotype of vaginal isolates from 4 to 6 mice was determined at selected time points. GC were recovered by vaginal swab for an average of 10.7 (range 7-14) and 7.3 (range 5-11) days. Three phases of Opa protein expression were defined. Within 1 to 2 days post inoculation (early phase), a high percentage of Opa1-expressing GC were recovered as compared to that of the inoculum from the majority (>75%) of mice. At mid-phase of infection (days 4-7), a decreased recovery of Opa-positive variants, which paralleled a decrease in the total number of GC recovered, was observed in 50-75% of infected mice. In 2 of 3 mice in which infection persisted for longer than 9 days, an increased recovery of Opa-positive GC, which coincided with an increase in total recovery, occurred late in infection. Additionally, we also recovered GC expressing multiple Opa proteins simultaneously, with a larger percentage of multiple expressors recovered late in infection. To test if selection for or against certain Opa phenotypes might be responsible for the three observed phases, a chloramphenicol-resistant strain of FA1090 was engineered. This strain will allow us to follow Opa protein expression within a specific population throughout the course of experimental infection. An alternative explanation for the observed changes in Opa phenotype in vivo is that *opa* gene phase variation may be induced by host factors. An increase in *opa* phase variation might also contribute to the simultaneous expression of multiple Opa proteins by individual GC. To address this possibility, we have constructed an *opaB::phoA* translational fusion. This fusion will allow us to examine the rate of phase variation under a range of in vitro conditions, as well as during experimental infection.

POSTER # 36

ANALYSIS OF *PSEUDOMONAS AERUGINOSA ALGZ* EXPRESSION REVEALS *ALGT* (σ^{22}) STRICT DEPENDENCE.

April B. Sprinkle, Patricia J. Baynham, Deborah M. Ramsey, and Daniel J. Wozniak

Pseudomonas aeruginosa is a major respiratory tract pathogen in cystic fibrosis patients. Emergence of *P. aeruginosa* strains producing the exopolysaccharide alginate is correlated with a severe disease outcome. AlgZ, a ribbon-helix-helix DNA-binding protein, was shown to control alginate synthesis by binding to and activating *algD*, the first gene of the alginate operon. AlgZ DNA binding activity was not observed in strains with mutations in *algT*, encoding an alternative sigma factor (σ^{22}) that is also required for alginate gene expression. In this study, an analysis of *algZ* regulation was undertaken. Strains were constructed which harbored mutations in *algT*, *algZ*, or *algT* and *algZ*. AlgZ-specific antiserum was generated and demonstrated that AlgZ is expressed in all mucoid *P. aeruginosa* CF isolates examined but not in nonmucoid CF-derived *P. aeruginosa* strains. This indicates that AlgZ-mediated control of alginate production is conserved among CF isolates. Primer extension studies showed that AlgZ-mediated control of *algD* was at the previously mapped *algD* promoter and confirmed that *algZ* was absolutely required for *algD* transcription. Electrophoretic mobility shift assays (EMSA) and Western blot analysis revealed that the expression of *algZ* was dependent on σ^{22}. These data were confirmed using primer extension to map the *algZ* transcription start site. The promoter sequence obtained agreed with a previous consensus of σ^{22}-regulated promoters and was further supported by mutagenesis of the putative -10 and -35 elements of the *algZ* promoter. Analysis of strains harboring *algZ::lacZ* fusions confirmed that σ^{22} was required for expression of *algZ*. Taken together the data suggest that expression of *algZ* is likely directly dependent on the alternative sigma factor σ^{22}.

POSTER # 37

ELEVATED MUTATION RATES IN *NEISSERIA GONORRHOEAE*: ASSOCIATION WITH ALTERATIONS IN DNA REPAIR AND REPLICATION GENES.

Sharon Taft-Benz, Diana Johannsen, JoAnn Dempsey and Janne Cannon

Neisseria gonorrhoeae, the causative agent of the sexually transmitted disease gonorrhea, is a gram-negative diplococcus that contains abundant repetitive DNA and an unusually high number of genes that phase vary by either insertion or deletion mutations occurring in simple repeats. Many of the phase variable genes encode surface proteins or affect probable virulence-related functions. A reduction in the fidelity of DNA replication or efficiency of DNA repair may result in increased rates of global mutation, or perhaps in selective increases in phase variation in simple repeat-containing genes. If *N. gonorrhoeae* could modulate the mutation rates of phase variable genes, the organism may adapt more quickly to the host environment and potentially increase the ability of the gonococcal population to survive. In order to assess the role of replication fidelity and DNA repair efficiency in the phase variation of neisserial genes, we first determined if *N. gonorrhoeae* contained DNA replication and DNA repair genes and systems similar to those found in *E. coli*, and if these genes were functional in DNA replication and repair. **Mismatch Repair (MMR):** The *N. gonorrhoeae* genome contains homologs of *mutS*, *mutL* and *uvrD* but not *mutH*. Knockout mutants lacking expression of either MutS or MutL exhibited increased frequencies of mutation to rifampicin and nalidixic acid resistance, as well as an elevated rate of frameshift mutations in the poly-G run in the *hpuA* gene, which encodes part of the hemoglobin receptor. The magnitude of the increases in the different types of mutations was less than those observed in corresponding *E. coli* mutants. **Very Short Patch (VSR) Repair:** Loss of VSR through insertion of stop codons in *vsr* did not result in a mutator phenotype in either a MMR-proficient or MMR-deficient *(ΔmutS)* background. **DNA Replication Fidelity:** In *E. coli*, substitution of certain conserved residues of the proofreading exonuclease subunit (encoded by the *dnaQ* gene) of DNA polymerase III result in dramatic increases in mutant frequency. Gonococcal mutants with similar substitutions in DnaQ showed only modest differences from the wild-type in the frequency of base substitution and frameshift mutations. Together, these data suggest that *Neisseria gonorrhoeae* utilizes a proofreading exonuclease and mismatch repair to maintain replication fidelity and repair DNA damage but that these systems may function with lower efficiency in *Neisseria gonorrhoeae* than in *E. coli*.

POSTER # 38

MOLECULAR GENETICS TOOLS FOR RECOMBINATION AND MUTAGENESIS IN *CLOSTRIDIUM PERFRINGENS*

John J. Varga and Stephen B. Melville

Clostridium perfringens is a Gram-positive anaerobic pathogen that causes a wide array of gastrointestinal diseases, including approximately 250,000 cases of acute food poisoning (AFP) annually in the United States. *C. perfringens* produces an enterotoxin (CPE) that is responsible for the symptoms associated with AFP. Previous research in the field has demonstrated that CPE is only produced by sporulating cells and that it is genetically co-regulated with sporulation, however, the nature of this linkage is unknown due in part to a lack of genetic tools. There are currently two mutagenesis strategies under investigation in our laboratory. The first strategy utilizes a random transposon mutagenesis system, based on the *mariner* transposon from *Drosophila* and the TN4351 transposon from *Bacteroides fragilis,* to generate a population of strains that will be screened for the ability to sporulate and to produce CPE. Preliminary work is underway using both transposons. The second mutagenesis system is a allelic replacement system using the *Escherichia coli gusA* gene as a lethal marker. GusA converts the substrate para-nitrophenyl-_-D-glucuronide to glucuronide and para-nitrophenol, a substance toxic to *C. perfringens*. The strain being used, SM101, has very low levels of endogenous GusA activity. By adding a functional *gusA* gene, bacteria that have undergone a second recombination event, in order to avoid producing the toxic compound, will be selected. The double-crossover system has been tested using the sporulation sigma-factor gene, *sigK*, and has generated two strains that demonstrate the expected phenotypes of a *sigK* gene knockout. Furthermore, the double-crossover strains produce undetectable levels of heat-stable spores and possess wild-type levels of GusA activity.

POSTER # 39

RELATEDNESS OF O ANTIGEN GENETIC LOCI BETWEEN *PSEUDOMONAS AERUGINOSA* AND *BURKHOLDERIA CEPACIA* WITH IDENTICAL O ANTIGEN SUBUNIT STRUCTURES

Arlene D. Vinion-Dubiel, Charles R. Dean, and Joanna B. Goldberg
Department of Microbiology, University of Virginia, Charlottesville, VA

Pseudomonas aeruginosa and *Burkholderia cepacia* complex (formerly *Pseudomonas cepacia*) are Gram-negative bacteria found in the environment that can also colonize the lungs of cystic fibrosis patients. Lipopolysaccharide of Gram-negative organisms contains three components: lipid A, core oligosaccharide, and O antigen, which consists of repeating subunits of 2-5 sugar residues. The O antigen subunits of *P. aeruginosa* serogroup O17 and *B. cepacia* serotype O5 are identical with the structure [-3)-β-D-ManNAc-(1-4)-α-L-Rha-(1-]. As these bacteria are phenotypically similar and occupy the same niche, we hypothesize that the O antigen genetic loci are related. A genomic DNA library from *B. cepacia* serotype O5 strain PC222 was screened for the ability to induce expression of the serotype O5 O antigen in *E. coli*, and one plasmid, 222-20 was isolated and sequenced. Additionally, a *P. aeruginosa* serogroup O17 strain, PAO17, was mutated by transposon insertion and a strain unable to express O antigen was isolated. Gene fragments from PAO17 containing the transposon were isolated and sequenced. Analysis of the gene sequences from these two bacteria revealed similarity to genes in other bacteria that are responsible for O antigen synthesis. Both O antigen loci from *B. cepacia* PC222 and *P. aeruginosa* PAO17 contain genes necessary for biosynthesis of rhamnose (*rmlA-D*), and two genes which together encode for a complete ABC transporter, likely responsible for export of the O antigen. While these two loci contain genes encoding for the same functions, the organization of the genes within the loci are dissimilar. For example, in *B. cepacia* PC222, the *rml* genes are in order *rmlBACD* while in *P. aeruginosa* PAO17, the order is *rmlBDAC*. Comparison of individual genes reveals that the ABC transporter genes within *P. aeruginosa* PAO17 and *B. cepacia* PC222 are more similar to one another than to the closest homologues in the sequenced strains *P. aeruginosa* PAO1 and *B. cepacia* J2315. The implications of these studies within the context of evolution of the O antigen genetic loci will be discussed.

POSTER # 40

IDENTIFICATION OF A SIGMA-22 INDEPENDENT PROMOTER FOR MUCD, AN HTRA(DEGP) PROTEASE HOMOLOGUE IN *PSEUDOMONAS AERUGINOSA*

Lynn F. Wood* and Dennis E. Ohman
Medical College of Virginia Campus of Virginia Commonwealth University, and McGuire Veterans Affairs Medical Center, Richmond, VA

HtrA(DegP)-like endoserine proteases, which typically protect the bacterial cell from environmental stresses (e.g., heat shock) have been found in a wide variety of bacteria. HtrA can also be required for pathogenesis. In *E. coli*, the promoter for *htrA(degP)* is up-regulated by heat shock and is under the control of the extracytoplasmic (ECF) sigma-E and a 2-component regulator, CpxR. The genomic sequence analysis of *Pseudomonas aeruginosa* shows that it has only two of these HtrA homologues, called MucD and AlgW, encoded by unlinked loci on its 6.2 Mb chromosome. Both MucD and AlgW have been associated with the production of the exopolysaccharide alginate, a virulence factor of *P. aeruginosa* involved in chronic pulmonary disease in cystic fibrosis (CF) patients. We have been investigating the expression of *mucD*, which is the fifth gene in the *algT(algU)-mucABCD* operon. The *algT* gene encodes an ECF sigma factor called sigma-22 that is required for expression of the *algD* operon encoding the enzymes for alginate biosynthesis. A *mucD* mutant was shown to produce alginate, but only under certain stress conditions. We performed a Western blot analysis of total cellular proteins, which showed that MucD was expressed well in both a typical nonmucoid strain (PAO1) and in a typical mucoid CF strain (FRD1). Although a polar insertion in *algT* was expected to severely block expression of the downstream *mucD* gene, the Western blot analysis showed that MucD was still produced, suggesting that *mucD* has its own promoter internal to the operon (here called P*mucD*). To locate the region containing P*mucD*, *mucD-lacZ* fusion constructs were built with increasing lengths of DNA 5' to the *mucD* coding region. Analysis of relative beta-galactosidase activities from the *mucD-lacZ* constructs localized the *mucD* promoter to a region between 220 and 330 bp upstream of the *mucD* start codon. Thus, P*mucD* actually lies within the *mucC* gene, which is relatively uncharacterized. Primer extensions were performed and the transcriptional start site of P*mucD* was identified in this region. The P*mucD* promoter shows some homology to sigma-70 promoters, but not to sigma-22. To compare the relative contributions of P*algT* and P*mucD* to *mucD* expression, a *mucD-lacZ* fusion was integrated into the chromosome of PAO1 and FRD1 strains, and the effect of polar insertions in *algT* was evaluated in each. This showed that approximately 40% of the total *mucD* transcriptional activity was due to the internal P*mucD* promoter. We hypothesized that P*mucD* was under independent control, possibly associated with stress. Initial studies suggested that P*mucD* expression was not autoregulated by expression of *mucD* or *algT* (sigma-22). Although *mucD* mutants were heat-sensitive, heat shock treatments did not affect P*mucD-lacZ* expression. Additional studies are in progress to identify potential regulators of P*mucD*.

POSTER # 41

HUMAN MONOCYTES AND NEUTROPHILS CAN KILL *CLOSTRIDIUM PERFRINGENS* UNDER AEROBIC AND ANAEROBIC CONDITIONS ONLY IF COMPLEMENT IS PRESENT

Michael E. Woodman and Stephen B. Melville
Department of Biology, Virginia Tech, Blacksburg, Virginia.

Clostridium perfringens is the causative agent of gas gangrene (clostridial myonecrosis). Polymorphonuclear leukocytes (PMNs) and monocytes are phagocytic cells that circulate in the blood and are recruited into the tissue during an infection. Previous research in the lab has shown that no mouse macrophages, including J774-33, mouse peritoneal and bone marrow derived macrophages could kill *C. perfringens* under aerobic or anaerobic conditions. Therefore, we wanted to determine if human PMNs or monocytes have the ability to kill *C. perfringens* under aerobic and anaerobic conditions. PMNs and monocytes alone were unable to kill *C. perfringens* under aerobic and anaerobic conditions. From these results, we wanted to determine if there was something present in human blood that was necessary for the killing of *C. perfringens*. When human serum was added to the PMNs and monocytes *C. perfringens* was killed approximately100 times more effectively than when serum was absent. However, when the serum was heat inactivated (i.e. no complement) the bacteria were not killed by the PMNs or monocytes. These results showed that complement was necessary for the killing of *C. perfringens*. However, in the absence of PMNs and monocytes, serum alone was unable to kill *C. perfringens* probably because it is a Gram-positive bacteria. To determine if phagocytosis is needed to kill *C. perfringens*, we added cytochalasin D to the samples to block phagocytosis. These results showed approximately ten-fold less killing in the presence of cytochalasin D indicating that phagocytosis is necessary to kill *C. perfringens*. To determine if the oxidative burst is required to kill *C. perfringens,* we added superoxide dismutase (SOD) and catalase, which scavenge oxygen radicals, to the cell samples. These results showed about five to ten fold less killing of the bacteria indicating that the oxidative burst does play a role in the killing of *C. perfringens* by PMNs and monocytes. We also wanted to determine the ratio of intracellular to extracellular bacteria inside PMNs and monocytes. This showed that about sixty percent of PMNs had intracellular bacteria, compared to monocytes with thirty percent.

POSTER # 42

SIALYLATION OF *NEISSERIA GONORRHOEAE* LIPOOLIGOSACCHARIDE (LOS) OCCURS DURING EXPERIMENTAL MURINE GENITAL TRACT INFECTION BUT IS NOT REQUIRED FOR INFECTION

Hong Wu and Ann E. Jerse
Department of Microbiology and Immunology, Uniformed Services University
Bethesda, Maryland 20814-4799

Introduction: Gonococci (GC) in urethral exudates demonstrate resistance to killing by normal human serum (NHS), which is lost by treatment with neuraminidase (NANase) or upon subculture. Serum resistance can be restored by incubation with cytidine monophosphate neuraminic acid (CMP-NANA). This unstable serum resistance results from the addition of sialic acid to the terminal N-lactose-N-tetraose molecule present on some gonococcal LOS species. Host sialic acid is used as the substrate in vivo, and the reaction is catalyzed by gonococcal sialyltransferase. In addition to conferring increased serum resistance, LOS sialylation increases resistance to opsonophagocytosis and to bactericidal activity of porin-specific antibodies. Collectively, these observations suggest that LOS sialylation confers a survival advantage to GC. Here we tested the requirement for sialyltransferase in *N. gonorrhoeae* lower genital tract infection using the estradiol-treated mouse infection model.

Methods and Results: The sialyltransferase (*lst*) gene of *N. gonorrhoeae* strain MS11 was cloned and mutated via the insertion of a nonpolar kanamycin resistance cassette (*aphA-3*). A sialyltransferase-deficient mutant (GP300) was constructed by allelic exchange. The *lst* mutant was functionally deficient for LOS sialylation in serum bactericidal and opsonophagocytosis assays. Specifically, wild type MS11 was more resistant to NHS when cultured in the presence of CMP-NANA; serum resistance was reduced after incubation with NANase (bactericidal$_{50}$ titers: wild type, 3%; wild type + CMP-NANA, >16%; wild type + CMP-NANA + NANase, 4%). In contrast, mutant GP300 was equally sensitive to NHS regardless of growth conditions (bactericidal$_{50}$ titers: 3-4% for all conditions). Wild type MS11 was significantly killed by polymorphonuclear leukocytes (PMNs) from both untreated and estradiol-treated mice ($p < 0.005$ and < 0.05 respectively), unless cultured in the presence of CMP-NANA. Mutant GP300 was also significantly killed by murine PMNs ($p < 0.05$). As expected, growth of GP300 in the presence of CMP-NANA did not protect from opsonophagocytic killing. To determine if LOS sialylation occurs during experimental murine infection, the serum sensitivity of wild type and Lst-deficient GC in vaginal suspensions from infected mice was measured. GC within vaginal suspensions from mice infected with wild type MS11 were resistant to NHS, and incubation with NANase or subculture to artificial media resulted in serum sensitivity. In contrast, mutant GP300 within vaginal suspensions was serum sensitive. To assess the role of gonococcal sialyltransferase in infection, estradiol-treated BALB/c mice were inoculated intravaginally with 10^6, 10^5 or 10^4 wild-type MS11 or mutant GP300 bacteria. No significant difference in duration of recovery or bacterial load was detected. In competitive (mixed) infection experiments, however, the relative recovery of the *lst* mutant compared to the wild type strain was dramatically reduced.

Conclusions: Although LOS sialylation protects GC from opsonophagocytosis by murine PMNs in vitro, no difference in the infectivity of the *lst* mutant or wild type strain was detected. Results from competitive infection experiments suggest that LOS sialylation provides a survival advantage in vivo. Introduction of an intact copy of the *lst* gene into mutant GP300 is underway to test if recovery during mixed infections can be restored to wild type levels through complementation *in trans*

POSTER # 43

GROUP SPECIFIC AND FUNCTIONAL DOMAINS OF AGRB, A TRANSMEMBRANE PROTEIN THAT IS ESSENTIAL FOR THE PROCESSING OF AGRD TO PRODUCE A SIGNAL PEPTIDE OF THE QUORUM SENSING SYSTEM THAT COORDINATELY CONTROLS THE EXPRESSION OF VIRULENCE FACTORS IN *STAPHYLOCOCCUS AUREUS*

Linsheng Zhang* and Guangyong Ji
Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814

The accessory gene regulator (*agr*) is the central regulatory system that controls the virulence gene expression in *Staphylococcus aureus*. This global regulatory locus consists of two transcripts: RNAII and RNAIII. RNAII encodes four genes (*agrA*, *B*, *C*, and *D*) whose gene products assemble a quorum sensing system: AgrA and AgrC resembles a two-component signal transduction system, and AgrB and AgrD are required to produce a signal molecule (autoinducing peptide, or AIP). Upon activation, this quorum sensing system positively regulates the transcription of RNAII as well as RNAIII that is the actual effecter of the Agr response. Four groups of *S. aureus* have been identified based on the *agr* sequences and the group specific interactions between AIP and AgrC, and between AgrB and AgrD. AgrB is a transmembrane protein and is involved in the proteolytic processing of AgrD propeptide. Transmembrane topology analysis of AgrB by PhoA fusions in *Escherichia coli* reveals that it contains six transmembrane segments that consist of four hydrophobic regions and two hydrophilic regions with several highly positively charged amino acid residues. In this study, a series of chimeric AgrBs were constructed between group I and group II AgrBs and these mutants were used to analyze the group specific domain(s) in AgrB that was responsible for AgrD processing. Our results revealed that the first transmembrane domain of group I AgrB was crucial in the specific processing of group I AgrD, however, the AgrB domains determined the group specificity for processing group III AgrD required a broader area than the first transmembrane domain from group I AgrB though wild-type group I AgrB could process group III AgrD. In contrast, the two hydrophilic domains of group II AgrB played an important role in the group specific processing of group II AgrD. We also found that several chimeric AgrBs were capable of processing AgrD from all three groups, and the two highly conserved regions among AgrBs were critical for AIP production as revealed by site-directed mutagenesis analyses, suggesting that these AgrB homologues utilize the same or similar mechanism in the processing of AgrDs. AgrB has been proposed to be a novel protein with dual functions: an enzymatic activity that proteolytically processes AgrD propeptide at two processing sites and catalyses the formation of a thioester bond, and a peptide transporter facilitating the export of the mature AIP. Our results suggested that some active domains in AgrBs were group specific, however, others could be conserved for the same activities in all groups.

ATTENDEES

Alejandro Aballay
Duke University Medical Center
DUMC Box 3580
Durham, NC 27710
Phone: 919-681-6765
Fax: 919-684-2790

Rose Alcantara
East Carolina University School of Medicine
600 Moye Blvd / Biotech Bldg Room 115
Greenville, NC 27834
alcantarar@mail.ecu.edu
Phone: 252-816-3125
Fax: 252-816-3135

Craig Altier
North Carolina State University
4700 Hillsborough Road
Raleigh, NC 27606
craig_altier@ncsu.edu
Phone: 919-513-6274
Fax: 919-513-6464

Abey Bandara
Virginia Tech
1410 Prices Fork Road
Blacksburg, VA 24061-0342
abandara@vt.edu
Phone: 540-231-4200
Fax: 540-231-3426

Eileen Barry
University of Maryland, Baltimore
685 W Baltimore St
Baltimore, MD 21201
ebarry@umaryland.edu
Phone: 410-706-3703
Fax: 410-706-6205

Susanne Bauman
Duke University
DUMC Box 3711
Durham, NC 27710
sjb7@duke.edu
Phone: 919-684-3794
Fax: 919-684-8885

Afrin Begum
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
abegum@usuhs.lim
Phone: 301-295-9627
Fax: 301-295-3773

Bryan Bellaire
1501 Kings Highway
PO Box 33932
Shreveport, LA 71130
bbella@lsuhsc.edu
Phone: 318-675-4383
Fax: 318-675-5764

Karin Berling
Old Dominion University
Mills Godwin Building
Norfolk, VA 23529
kberl@odu.edu
Phone: 757-683-3613
Fax: 757-683-5283

Amy Bordner
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
abordner@usuhs.mil
Phone: 301-295-9627
Fax: 301-295-3773

Philip Boucher
CBER/FDA HFM-434 ; Bldg29, Rm 528
8800 Rockville Pike
Bethesda, MD 20892
boucher@cber.fda.gov
Phone: 301-496-1785
Fax: 301-402-2776

Amy Bouton
University of Virginia Health System
Box 800734
Charlottesville, VA 22908
ahb8y@virginia.edu
Phone: 434-924-2513
Fax: 434-982-1071

Stephen Boyle
CMMID
VMRCVM, Virginia Tech
Blacksburg, VA 24061-0342
smboyle@vt.edu
Phone: 540-231-4641
Fax: 540-231-3426

Miriam Braunstein
Campus Box 7290, School of Medicine
University of North Carolina at Chapel Hill
Chapel Hill, NC 27599-7290
braunste@med.unc.edu
Phone: 919-966-5051
Fax: 919-962-8103

Sandra Brown
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
sabrown@usuhs.mil
Phone: 301-295-9627
Fax: 301-295-3773

Tim Brown
East Carolina University School of Medicine
600 Moye Blvd / Biotech Bldg Room 115
Greenville, NC 27834
brownti@mail.ecu.edu
Phone: 252-816-3125
Fax: 252-816-3135

Eric Buckles
16 S. Pine Street
MBTF Bldg, Room 900
Baltimore, MD 21201
ebucklesphd@yahoo.com
Phone: 410-706-0151
Fax: 410-706-8700

Drusilla Burns
Building 29, Room 130; HFM-434
8800 Rockville Pike
Bethesda, MD 20892
burns@cber.fda.gov
Phone: 301-402-3553
Fax: 301-402-2776

M. Worth Calfee
East Carolina University
600 Moye Blvd
Greenville, NC 27834
MWC0306@mail.ecu.edu
Phone: 252-816-2351
Fax: 252-816-3535

Jodi Camberg
American Red Cross Holland Lab
15601 Crabbs Branch Way
Rockville, MD 20855
CambergJ@usa.redcross.org
Phone: 301-738-0466

Janne Cannon
University of North Carolina
804 M. E. Jones Bldg, CB#7290
Chapel Hill, NC 27599
jgc@med.unc.edu
Phone: 919-966-4774
Fax: 919-962-8103

Nicholas Carbonetti
University of Maryland Medical School
655 W. Baltimore Street / BRB 13-009
Baltimore, MD 21201
ncarbone@umaryland.edu
Phone: 410-706-7677
Fax: 410-706-2129

Wendy Carr
FDA/CBER/DBPAP Bldg 29, Room 201
HFM-440 8800 Rockville Pike
Bethesda, MD 20892
carrw@cber.fda.gov
Phone: 301-451-4252
Fax: 301-402-2776

Anissa Cheung
FDA/CBER 29 Lincoln Drive
Building 29, Rm 416
Bethesda, MD 20892
cheunga@cber.fda.gov
Phone: 301-402-4854
Fax: 301-402-2776

Gail Christie
Virginia Commonwealth University
PO Box 980678
Richmond, VA 23298-0678
christie@hsc.edu
Phone: 804-828-9093
Fax: 804-828-9946

Leah Cole
UNC Chapel Hill
804 Mary Ellen Jones Bldg, Campus
Box 7290
Chapel Hill, NC 27599
lecole@med.unc.edu
Phone: 919-966-2637
Fax: 919-962-8103

Peggy Cotter
Dept Molecular, Cellular, and Dev Biology
University of California, Santa Barbara
Santa Barbara, CA 93106
cotter@lifesci.ucsb.edu
Phone: 805-893-5176
Fax: 805-893-4724

Emily Crow
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
ecrow@usuhs.mil
Phone: 301-295-9627
Fax: 301-295-3773

Lynne Crowther
University of Maryland School of Medicine
10 S. Pine Street; MSTF 900
Baltimore, MD 21201
crowtherlynne@hotmail.com
Phone: 410-706-7560
Fax: 410-706-8700

Ano Daniel
University of Maryland School of Medicine
10 S. Pine Street; MSTF 900
Baltimore, MD 21201
adani002@umaryland.edu
Phone: 410-706-7560
Fax: 410-706-8700

Antonio Digiandomenico
1300 Jefferson Park Avenue
7231 Jordan Hall
Charlottesville, VA 22908
ad3b@virginia.edu
Phone: 434-243-2775
Fax: 434-982-1071

Song-ze Ding
University of Virginia, Box 800734
1300 JPA Avenue
Charlottesville, VA 22908
soding@virginia.edu
Phone: 434-243-2775
Fax: 434-982-1071

Michael Donnenberg
University of Maryland School of Medicine
10 S. Pine Street; MSTF 900
Baltimore, MD 21201
mdonnenb@umaryland.edu
Phone: 410-706-7560
Fax: 410-706-8700

Edward Dudley
University of Maryland - Baltimore
685 W Baltimore St Rm 447
Baltimore, MD 21201
edudl001@umaryland.edu
Phone: 410-706-7376
Fax: 410-706-6205

Paula Fernandes
University of Maryland School of Medicine
10 S. Pine Street; MSTF 900
Baltimore, MD 21201
pfern002@umaryland.edu
Phone: 410-706-7560
Fax: 410-706-8700

Aaron Firoved
12011 Twinbrook Pkwy
Rockville, MD 20851
afiroved@mail.nih.gov
Phone: 571-215-6850
Fax: 301-480-0326

David Fitzgerald
37 Convent Drive
Building 37, 5124
Bethesda, MD 20892
djpf@helix.nih.gov
Phone: 301-496-9457
Fax: 301-402-1969

Brenda Frankel
902 Stellar Chance Bldg
422 Curie Blvd
Philadelphia, PA 19104
bfrankel@mail.med.upenn.edu
Phone: 215-898-6107
Fax: 215-573-8052

Robert Fulcher
UNC - Chapel Hill School of Medicine
804 Mary Ellen Jones Bldg, Campus Box 7290
Chapel Hill, NC 27599-7290
rfulcher@med.unc.edu
Phone: 919-966-2637
Fax: 919-962-8103

Jorge Galán
Boyer Center for Molecular Medicine
Yale University School of Medicine
New Haven, CT 06536-0812
jorge.galan@yale.edu
Phone: 203-737-2404
Fax: 203-737-2630

Jason Gee
East Carolina University School of Medicine
600 Moye Blvd / Biotech Bldg Room 115
Greenville, NC 27834
geej@mail.ecu.edu
Phone: 252-816-3125
Fax: 252-816-3135

Carol Gilchrist
University of Virginia
MR4 Bldg, Rm 2115; 409 Lane Rd
Charlottesville, VA 22908
cg2p@virginia.edu
Phone: 434-924-5167
Fax: 434-924-0075

Joanna Goldberg
1300 Jefferson Park Avenue
7231 Jordan Hall
Charlottesville, VA 22908
jbg2b@virginia.edu
Phone: 434-243-2774
Fax: 434-982-1071

Kerian Grande
1217 East Marshall St
Medical Sciences Bldg, Rm 209
Richmond, VA 23298
kkgrande@hsc.vcu.edu
Phone: 804-628-0246
Fax: 804-828-9946

Lillian Gray
USUHS B4121
4301 Jones Bridge Road
Bethesda, MD 20814
lgray@usuhs.mil
Phone: 301-295-9620
Fax: 301-295-1545

Vanessa Grippe
FDA/CBER/DBPAP HFM-434
1401 Rockville Pike
Rockville, MD 20852
grippe@cber.fda.gov
Phone: 301-496-4288
Fax: 301-402-2776

Nanette Guyer
811 Mary Ellen Jones CB#7290
Dept Microbiology and Immunology
Chapel Hill, NC 27599
nbg83@med.unc.edu
Phone: 919-966-3663
Fax: 919-962-8103

Terri Hamrick
Campbell University School of Pharmacy
PO Box 1090
Buies Creek, NC 27506
hamrick@camel.campbell.edu
Phone: 910-893-1705
Fax: 910-893-1697

Emily Henderson
University of North Carolina
CB#7290, 804 MEJB
Chapel Hill, NC 27599-7290
henderse@dentistry.unc.edu
Phone: 919-966-2637

Erik Hewlett
University of Virginia School of Medicine
Charlottesville, VA 22908
eh2v@virginia.edu
Phone: 434-982-4334

Michael Hornback
East Carolina University, 600 Moye Blvd.
Biotech 117
Greenville, NC 27834
hornbackm@mail.ecu.edu
Phone: 252-816-3135
Fax: 252-816-3535

Krischan Hudson
University of Virginia
PO Box 800734
Charlottesville, VA 22908
KJH6N@virginia.edu
Phone: 434-924-9995
Fax: 434-982-1071

Molly Hughes
Division of Infectious Diseases
UVA Health Sciences System
Charlottesville, VA 22908
mah3x@cms.mail.virginia.edu
Phone: 434-243-9552

Thomas Inzana
Virginia Polytechnic Institute
1410 Prices Fork Road
Blacksburg, VA 24061
tinzana@vt.edu
Phone: 540-231-4692
Fax: 540-231-3426

Kara Jackson
300 Med Ctr Blvd, 5136 Gray Bldg
Microbiology and Immunology
Winston-Salem, NC 27157
kajackso@wfubmc.edu
Phone: 336-716-9333
Fax: 336-716-9928

Brian Janes
CBER/FDA HFM-440
8800 Rockville Pike
Bethesda, MD 20892
janes@cber.fda.gov
Phone: 301-451-4247
Fax: 301-402-2776

Ann Jerse
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
ajerse@usuhs.mil
Phone: 301-295-9627
Fax: 301-295-3773

Guangyong Ji
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
gji@usuhs.mil
Phone: 301-295-9621
Fax: 301-295-1545

Ali Johnson
Duke University
Box 3711 DUMC
Durham, NC 27710
ahp@duke.edu
Phone: 919-684-3794
Fax: 919-684-8885

Nicole Judge
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
njudge@usuhs.mil
Phone: 301-295-3405
Fax: 301-295-3773

Bob Kadner
University of Virginia
PO Box 800734
Charlottesville, VA 22908-0734
rjk@virginia.edu
Phone: 434-924-2532
Fax: 434-293-9829

Tom Kawula
CB# 7290, 804 MEJB
UNC-CM
Chapel Hill, NC 27599-7290
kawula@med.unc.edu
Phone: 919-966-9699
Fax: 919-962-8103

Nicole Kesty
Duke University
DUMC Box 3711
Durham, NC 27710
nck@duke.edu
Phone: 919-684-3794
Fax: 919-684-8885

Ryan Kruger
422 Curie Blvd
902 Stellar Chance Blvd
Philadelphia, PA 19104
kruger@mail.med.upenn.edu
Phone: 215-898-6107
Fax: 215-573-8052

Meta Kuehn
Duke University Medical Center
Box 3711 DUMC
Durham, NC 27710
meta.kuehn@duke.edu
Phone: 919-684-2545
Fax: 919-684-8885

Sherry Kurtz
University of North Carolina, CB 7290
Mary Ellen Jones Bldg Rm 816
Chapel Hill, NC 27514
skurtz@med.unc.edu
Phone: 919-966-5041
Fax: 919-962-8103

Holly Kuzmiak
Drew University
36 Madison Ave
Madison, NJ 07940
hkuzmiak@drew.edu
Phone: 973-408-4624

Gloria Lee
FDA/CBER 1401 Rockville Pike
HFM-434
Rockville, MD 20852
leeg@cber.fda.gov
Phone: 301-496-4289
Fax: 301-402-2776

Beth Leffel
USAMRIID
1425 Porter Street
Frederick, MD 21702-5011
elizabeth.leffel@amedd.army.mil
Phone: 301-619-4459
Fax: 301-619-6911

Mechelle Lesher
FDA/CBER
29 Lincoln Drive, Room 431
Bethesda, MD 20892
lesher@cber.fda.gov
Phone: 301-496-4776
Fax: 301-480-5527

Norma Liggett
East Carolina University; 600 Moye Blvd
Biotech #104
Greenville, NC 27834
liggettn@mail.ecu.edu
Phone: 252-744-3120
Fax: 252-744-3535

Jianquin Lin
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
jlin@usuhs.mil
Phone: 301-295-9620

Wensheng Luo
University of Maryland School of Medicine
10 S. Pine Street; MSTF 900
Baltimore, MD 21201
wluo001@umaryland.edu
Phone: 410-706-7560
Fax: 410-706-8700

Suzanne Lybarger
American Red Cross Holland Lab
Dept. Biochmestry; 15601 Crabbs
Branch Way
Rockville, MD 20855
lybargers@usa.redcross.org
Phone: 301-738-0470
Fax: 301-470-0740

Barbara Mann
University of Virginia
PO Box 801340
Charlottesville, VA 22908
bjm2r@virginia.edy
Phone: 434-924-9266
Fax: 434-924-0075

Richard Marconi
Virginia Commonwealth University
Dept Microbiology, PO Box 980678
Richmond, VA 23298-0678
rmarconi@hsc.vcu.edu
Phone: 804-828-3779
Fax: 804-828-9946

Amanda McBroom
Duke University Medical Center, Biochemistry
Box 3711 DUMC
Durham, NC 27710
ajm12@duke.edu
Phone: 919-684-3794
Fax: 919-684-8885

Justin McDonough
Campus Box 7290, School of Medicine
University of North Carolina at Chapel
Hill
Chapel Hill, NC 27599-7290
jmcdono@med.unc.edu
Phone: 919-966-5041
Fax: 919-962-8103

John McDowell
Virginia Commonwealth University
PO Box 980678
Richmond, VA 23298-0678
jvmcdowe@hsc.vcu.edu
Phone: 804-828-3779
Fax: 804-828-9946

Marian McKee
37 Convent Drive MSC 4264
37/5124
Bethesda, MD 20892-4264
mmckee@helix.nih.gov
Phone: 301-402-0241
Fax: 301-402-1969

Stephen Melville
Virginia Tech / Dept Biology
2119 Derring Hall
Blacksburg, VA 24061
melville@vt.edu
Phone: 540-231-14441
Fax: 540-231-9307

Tod Merkel
FDA/CBER Bldg 29, Rm 418
HFM-434
Bethesda, MD 20892
merkel@cber.fda.gov
Phone: 301-496-5564
Fax: 301-402-2776

Elenor Metcalf
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
emetcalf@usuhs.mil

Karen Meysick
FDA/CBER Bldg 29, Rm 423
HFM-434 8800 Rockville Pike
Bethesda, MD 20892
meysick@cber.fda.gov
Phone: 301-451-3952
Fax: 301-402-2776

Anastasia Mitchell
University of Virginia
Box 800734
Charlottesville, VA 22908
am9t@virginia.edu
Phone: 434-243-2775
Fax: 434-982-1071

Suman Mukhopadhyay
8705 Greenmead Drive
University of Maryland
College Park, MD 20742
mukhopad@umbi.umd.edu
Phone: 301-314-6812
Fax: 301-314-6855

Jean-Phillipe Nougayrede
University of Maryland School of Medicine
10 S. Pine Street; MSTF 900
Baltimore, MD 21201
jnoug001@umaryland.edu
Phone: 410-706-7560
Fax: 410-706-8700

Tadhg Ó Cróinín
FDA
29 Lincoln Drive
Bethesda, MD 20892
ocroinin@cber.fda.gov
Phone: 301-496-4289
Fax: 301-402-2776

David O'Brien
Virginia Tech / Dept Biology
2119 Derring Hall
Blacksburg, VA 24060
dobrien@vt.edu
Phone: 540-231-8937
Fax: 540231-9307

Colin O'Connell
University of Maryland School of Medicine
10 S. Pine Street; MSTF 900
Baltimore, MD 21201
Phone: 410-706-7560
Fax: 410-706-8700

Seth Ogden
University of Virginia; Room 7231 Jordan Hall
1300 Jefferson Park Avenue
Charlottesville, VA 22908
sro7f@virginia.edu
Phone: 434-243-2775
Fax: 434-982-1071

Dennis Ohman
Medical College of Virginia / PO Box 980678
1101 E. Marshall Street, Room 5-051
Richmond, VA 23298-0678
dohman@hsc.vcu.edu
Phone: 804-828-9728
Fax: 804-828-9946

Igor Olekhnovich
University of Virginia School of Medicine
PO Box 800734
Charlottesville, VA 22908-0734
io4n@virginia.edu
Phone: 434-924-9599
Fax: 434-982-1071

Paul Orndorff
North Carolina State University
4700 Hillsborough Road
Raleigh, NC 27606
paul_orndorfff@ncsu.edu
Phone: 919-513-6207
Fax: 919-513-6464

Manuel Osorio
CBER/FDA 29 Lincoln Drive
Building 29, Room 429; HFM-440
Bethesda, MD 20892
osorio@cber.fda.gov
Phone: 301-402-0730

Kate Owen
University of Virginia
Box 800734
Charlottesville, VA 22908
ko4q@virginia.edu
Phone: 434-294-9995

Sehmi Paik
521 N. 11th Street; Wood Bldg, Box 980566
Virginia Commonwealth University
Richmond, VA 23298-0566
smpaik@vcu.edu
Phone: 804-628-7009
Fax: 804-828-0150

James Paulley
East Carolina University, 600 Moye Blvd.
Biotech Bldg. 117
Greenville, NC 27858
jtp1003@mail.ecu.edu
Phone: 252-816-3135

Wuhong Pei
USUHS B4121; MIC/USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
wpei@usuhs.mil
Phone: 301-295-9620
Fax: 301-295-1545

Eb Pesci
East Carolina University
BT 132; ECU School of Medicine
Greenville, NC 27858
pescie@mail.ecu.edu
Phone: 252-816-2351
Fax: 253-816-3535

William Petri
University of Virginia, Div Infectious Diseases
P.O. Box 801340
Charlottesville, VA 22908-1340
wap3g@virginia.edu
Phone: 434-924-5621
Fax: 434-924-0075

Paul Phibbs
East Carolina University
600 Moye Blvd
Greenville, NC 27858
phibbspa@mail.ecu.edu
Phone: 252-816-2700
Fax: 252-816-3104

Zoe Phillips-Worthington
University of Maryland, Baltimore
685, W. Baltimore Street
Baltimore, MD 21201
zphil001@umaryland.edu
Phone: 410-706-0097
Fax: 410-706-2129

Alison Pickering
FDA, 29 Lincoln Drive, NIH Campus
HFM-4341
Bethesda, MD 20892
pickering@cber.fda.gov
Phone: 301-496-4289
Fax: 301-402-2776

Andrei Pomerantsev
30 Convent Drive MSC 4350
Bldg 30, Room 303
Bethesda, MD 20892-4350
apomeran@mail.nih.gov
Phone: 301-451-9817
Fax: 301-480-0326

Jessica Pritchard
2401 Arlington Blvd
Apt 4
Charlottesville, VA 22903
jep4n@virginia.edu
Phone: 434-295-0206

Girija Ramhkrisunan
University of Virginia
Building MR4, Room 2115
Charlottesville, NC 22908
gr6q@virginia.edu
Phone: 434-924-5167
Fax: 434-924-0075

Douglas Reed
USAMRIID
1425 Porter Street
Fort Detrick, MD 21702-5011
douglas.reed@amedd.army.mil
Phone: 301-619-6728
Fax: 301-619-6911

Steve Richardson
Wake Forest University School of Medicine
Medical Center Blvd.
Winston-Salem, NC 27157
srichard@wfubmc.edu
Phone: 336-716-4275
Fax: 336-716-9928

Edson Rocha
East Carolina University
600 Moye Blvd
Greenville, NC 27858
rochae@mail.ecu.edu
Phone: 252-816-3126
Fax: 252-816-3535

Marty Roop
East Carolina University School of Medicine
600 Moye Blvd
Greenville, NC 27858-4354
roopr@mail.ecu.edu
Phone: 252-816-1357
Fax: 252-816-3535

John Rose
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
jrose@usuhs.mil
Phone: 301-295-3424
Fax: 301-295-1545

John Roth
University of Utah, Dept Biology
257 South 1400 East
Salt Lake City, UT 84112
roth@bioscience.utah.edu

Christelle Roux
University of Louisiana
300 E. St. Mary Blvd, Room 108
Lafayette, LA 70504
cmr2862@louisiana.edu
Phone: 337-482-1310
Fax: 337-482-5660

Chad Roy
USAMRIID
1425 Porter Street
Fort Detrick, MD 21702
chad.roy@amedd.army.mil
Phone: 301-619-6722
Fax: 301-619-2348

Craig Roy
Boyer Center for Molecular Medicine
Yale University School of Medicine
New Haven, CT 06536
craig.roy@yale.edu
Phone: 203-737-2408

Laura Runyen-Janecky
Gottwald Science Center
University of Richmond
Richmond, VA 23173
lrunyenj@richmond.edu
Phone: 804-287-6390
Fax: 804-289-8233

Surashri Sahu
8075 Greenmead Drive
University of Maryland
College Park, MD 20742
mukhopad@umbi.umd.edu
Phone: 301-314-6812
Fax: 301-314-6855

Maria Sandkvist
American Red Cross Holland Lab
15601 Crabbs Branch Way
Rockville, MD 20855
sandkvist@usa.redcross.org
Phone: 301-738-0608
Fax: 301-738-0740

Maria Scott
American Red Cross Holland Lab
15601 Crabbs Branch Way
Rockville, MD 20855
scottmaria@usa.redcross.org
Phone: 301-738-0461

Hua Shen
Lockett Hall, Room 214
PO Box 9064
Petersburg, VA 23806
hshen@vsu.edu
Phone: 804-524-6991
Fax: 804-524-5732

C. Jeffrey Smith
East Carolina University
600 Moye Blvd
Greenville, NC 27834
smithcha@mail.ecu.edu
Phone: 252-816-3127
Fax: 252-816-3535

Cheryl Spence
East Carolina University
600 Moye Blvd
Greenville, NC 27834
cs0202@mail.ecu.edu
Phone: 252-816-3365

April Sprinkle
Wake Forest University Health Sciences
Medical Center Blvd, 5136 Gray Bldg
Winston-Salem, NC 27157
asprinkl@wfubmc@edu
Phone: 336-716-9333
Fax: 336-716-9928

Scott Stibitz
CBER/FDA, NIH Bldg 29 / Rm 514B
8800 Rockville Pike HFM-440
Bethesda, MD 20892
stibitz@cber.fda.gov
Phone: 301-827-5156
Fax: 301-402-2776

William Swords
Wake Forest University School of Medicine
5101 A Gray Building / Medical Center Blvd
Winston-Salem, NC 27157
wswords@wfubmc.edu
Phone: 336-713-5049
Fax: 336-716-9928

Sharon Taft-Benz
University of North Carolina / Microbiology
Room 811 MEJB, CB#7290
Chapel Hill, NC 27599
sharon_taft-benz@med.unc.edu
Phone: 919-966-3663
Fax: 919-962-8103

Louise Temple
Drew University
36 Madison Ave
Madison, NJ 07940
ltemple@drew.edu
Phone: 973-408-3119
Fax: 973-408-3847

Mangu Tyasi
Drew University
36 Madison Ave
Madison, NJ 07940
Phone: 973-408-3119

Teresa Urban
University of Virginia
1300 Jefferson Park Avenue
Charlottesville, VA 22908
turb0@virginia.edu
Phone: 434-243-2775
Fax: 804-982-1071

Michelle Valderas
East Carolina University
600 Moye Blvd
Greenville, NC 27834
lafleur28@yahoo.com
Phone: 252-816-3125
Fax: 252-816-3535

Raphael Valdivia
Duke University
273 Jones Building
Durham, NC 27710
valdi001@mc.duke.edu
Phone: 919-668-3831
Fax: 919-681-9193

Monique van Hoek
George Mason University
MS 4E3
Manassas, VA 20110
mvanhoek@gmu.edu
Phone: 703-993-4273
Fax: 703-993-4288

John Varga
Virginia Tech / Biology
2119 Derring Hall
Blacksburg, VA 24060
jvarga@vt.edu
Phone: 540-231-8937
Fax: 540-231-9307

Arlene Vinson-Dubiel
University of Virginia
PO Box 800734
Charlottesville, VA 22908-0734
av5z@virginia.edu
Phone: 434-243-2775
Fax: 434-982-1071

Doug Warner
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
dwarner@usuhs.mil
Phone: 301-295-9627
Fax: 301-295-3773

Jennifer Wolf
University of Virginia
1300 Jefferson Park Avenue; Room 7231
Charlottesville, VA 22908
jbuck1224@hotmail.com
Phone: 434-243-2775
Fax: 434-982-1071

Xiaolin Wong
University of Maryland School of Medicine
10 S. Pine Street; MSTF 900
Baltimore, MD 21201
Phone: 410-706-7560
Fax: 410-706-8700

Lynn Wood
PO Box 980678
1217 E. Marshall Street; MSB Room 209
Richmond, VA 23298
alwood@hsc.vcu.edu
Phone: 804-628-0246
Fax: 804-829-9946

Michael Woodman
Virginia Tech
2119 Derring Hall
Blacksburg, VA 24061
mwoodman@vt.edu
Phone: 540-231-1441
Fax: 540-231-9307

Daniel Wozniak
Microbiology and Immunology
Wake Forest University, Med Ctr Blvd
Winston-Salem, NC 27109
dwozniak@wfubmc.edu
Phone: 336-716-2016
Fax: 336-716-9928

Hong Wu
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
hwu@usuhs.mil
Phone: 301-295-9627
Fax: 301-295-3773

Mei-Shin Yang
FDA/CBER/DBPAP Bldg 29, Room 526
29 Lincoln Drive
Bethesda, MD 20892
yang@cber.fda.gov
Phone: 301-496-1785
Fax: 301-402-2776

Linsheng Zhang
USUHS
4301 Jones Bridge Road
Bethesda, MD 20814
lizhang@usuhs.mil
Phone: 301-295-9620

Steven Zink
N29 RM B7
8800 Rockville Pike
Bethesda, MD 20892
zinks@cber.fda.gov
Phone: 301-496-4289
Fax: 301-402-2776